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**THE EFFECT OF MALATHION ON THE ACTIVITY AND PERFORMANCE  
OF ACTIVATED SLUDGE**

THESIS

Erik G. Rauglas, Captain, USAF

AFIT-ENV-MS-15-M-197

**DEPARTMENT OF THE AIR FORCE  
AIR UNIVERSITY**

***AIR FORCE INSTITUTE OF TECHNOLOGY***

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**Wright-Patterson Air Force Base, Ohio**

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AFIT-ENV-MS-15-M-197

THE EFFECT OF MALATHION ON THE ACTIVITY AND PERFORMANCE OF  
ACTIVATED SLUDGE

THESIS

Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the  
Degree of Master of Science in Engineering Management

Erik G. Rauglas, BS

Captain, USAF

March 2015

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THE EFFECT OF MALATHION ON THE ACTIVITY AND PERFORMANCE OF  
ACTIVATED SLUDGE

Erik G. Rauglas, BS  
Captain, USAF

Committee Membership:

Dr. Willie F. Harper Jr.  
Chair

Dr. Daniel L. Felker  
Member

Lt Col LeeAnn Racz, PhD  
Member

Maj Gregory D. Hammond, PhD  
Member

## Abstract

Decontamination activities following a chemical warfare agent (CWA) incident may generate significant quantities of contaminated wash water. The disposal method identified for this water will be specific to each location, with some communities choosing to utilize the local wastewater treatment plant to process the waste. This raises concerns about the effects of CWAs on treatment facilities, which utilize biological wastewater treatment methods. These facilities serve as an important line of defense against the spread of pollutants to the aquatic environment. The presence of CWAs in the influent stream may inhibit the microbial action that is responsible for remediating contaminated wastewater.

The goal of the current study was to evaluate the effect of malathion on the activity and performance of activated sludge bioreactors. Malathion is one of many organophosphate (OP) based pesticides and is considered a well-rounded surrogate for *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX), an OP based CWA. This study employed respirometry, short term batch tests, and long-term exposure experiments to investigate the effects of different concentrations of malathion on activated sludge performance.

Respirometry results showed that the maximum respiration rates were approximately  $45 \mu\text{g O}_2 \text{ min}^{-1}$  when the sludge was not exposed to malathion. However, when malathion was added over a range of concentrations between  $0.1 \mu\text{g L}^{-1}$  and  $5 \text{ mg L}^{-1}$ , the maximum respiration rates varied between 33 and  $59 \mu\text{g O}_2 \text{ min}^{-1}$ . The oxygen consumption curves were similar in each case, beginning with rapid oxygen

consumption for the initial 2.5 – 3 hours, followed by a gradual, nonlinear decline in the respiration rates until the experimental time reached approximately 5 hours when the respiration rates were generally between 5 – 15  $\mu\text{g O}_2 \text{ min}^{-1}$ .

Short-term batch tests showed that chemical oxygen demand (COD) removal was not negatively impacted by the presence of malathion concentrations of 0.1 or 3  $\text{mg L}^{-1}$ . However, ammonia removal was slowed by the presence of malathion at both 0.1 and 3  $\text{mg L}^{-1}$  with a positive correlation of the removal rate to the quantity of total suspended solids. Despite variations in the ammonia removal rate, the final ammonia concentrations were unaffected by the presence of malathion at both 0.1 or 3  $\text{mg L}^{-1}$  when compared against the control. Long-term exposure experiments demonstrated that both COD and ammonia removal were negatively affected at concentrations of 3.0  $\text{mg L}^{-1}$  and unaffected at concentrations of 0.1  $\text{mg L}^{-1}$ .

Short-term exposure to malathion is unlikely to interrupt microbial respiration, COD removal, or nitrification in the range of concentrations tested in this study. However, long-term exposure to malathion has the potential to negatively impact COD removal and nitrification processes at or above 3.0  $\text{mg L}^{-1}$ .

*For my girls*



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This work would not have been possible without the constant support and guidance of many people. First, I would like to thank Dr. Willie Harper for his extraordinary guidance and mentoring throughout my time at AFIT. You kept pushing to bring the best out in everything I did. To Dr. Daniel Felker, your efforts in helping me with method development and sample analysis on both the HPLC and the GC/MS brought another level to my research that would not have been possible without your support. Despite my best efforts at running the AFIT GC/MS into the ground, your skill and patience ensured that every time something went wrong, be it inlet liners cracking from thermal stress or auto sampler needles being worked to failure, the equipment was quickly repaired and operational with minimal impact to what became a very tight schedule. To SMSgt Seth Martin and Kandace Bailey, I could not have asked for a better set teammates or friends. With your efforts, we were able to achieve much more than would have been possible alone. Lastly, I want to thank my family for providing me with the love and support that kept me going. My wife, who I am especially grateful to for giving birth to our daughter and taking on a disproportionate amount of the parenting load, which allowed me to focus during my coursework. My daughter, who constantly reminded me that it is important to enjoy the journey and not solely focus on the finish. Without all of you, this journey would have been very different and not nearly as much fun.

Erik G. Rauglas

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# **THE EFFECT OF MALATHION ON THE ACTIVITY AND PERFORMANCE OF ACTIVATED SLUDGE**

## **I. Introduction**

### **History of Chemical Weapons**

Man has always been looking for ways to generate an advantage for themselves on the battlefield. The development and use of chemical warfare agents has been an integral part in this effort. Chemical weapons, as we think of them today, made their first appearance in WWI. The first gas attack in WWI took place in Ypres, 22 April 1915, when the Germans discharged 5,730 cylinders of chlorine gas along a 6 km front (Spiers, 2010; Maynard, 2007). Despite their first large industrial scale use in WWI, chemical weapon usage dates back as far as 2000 B.C.E. (Coleman, 2005). Examples include ancient myths of armors dipped in venom, poisoned water, plagues, secret formulas, and combustible weapons. Specific examples of these weapons can be found in Greek myths starting in 750 B.C.E., as well as in descriptions of Greek fire by Byzantine, and Islamic sources in the 7<sup>th</sup> to 14<sup>th</sup> century C.E. (Salem, Ternay Jr., & Smart, 2008).

During WWI, chlorine gas was the first chemical agent used by Germany on a large scale. It was first recognized as a potential asphyxiating agent by Swedish chemist Karl Wilhelm Scheele in 1774 (Salem, Ternay Jr., & Smart, 2008). Like chlorine gas, many of the other chemical weapons were used during WWI. Many were discoveries of the 18<sup>th</sup> and 19<sup>th</sup> century; hydrogen cyanide in 1782, cyanogen chloride in 1802, phosgene in 1812, mustard in 1822, and chloropicrin in 1848 (Salem, Ternay Jr., &

Smart, 2008). During WWI, 125 K tons of gas was used. This produced 1.32 M casualties, 1.2 M wounded and 91 K killed (Salem, Ternay Jr., & Smart, 2008) which was a relatively small percentage when compared to the approximate 8.5 M killed during the war (Showalter, 2014).

From the beginning of WWI until 1936, phosgene and mustard gas were considered the most dangerous chemical weapons. On 23 Dec 1936, while continuing research that began in 1934 looking for new insecticides, Dr. Gerhard Schrader of I.G. Farben in Germany accidentally isolated the first organophosphate nerve agent, tabun, which was later designated by the United States as GA (Salem, Ternay Jr., & Smart, 2008). Two years after the discovery of tabun, Dr. Schrader, along with Ambrose, Rudriger, and Van Der Linde working at I.G. Farben, synthesized sarin, which was designated Trilon-46 or T-144 by Germany and designated as GB by the United States. During the latter part of WWII, in 1944, Germany discovered soman (GD) which combines the features of both sarin and tabun (Salem, Ternay Jr., & Smart, 2008; Kikilo, Fedorenko, & Ternay Jr., 2008).

Following WWI, public and governmental opinion had shifted away from the use of chemical weapons. Despite superiority in the field of chemical weapons, their potential for providing a significant advantage and having produced 12,000 tons of tabun, Germany chose not to use their chemical weapon stockpile during WWII (Maynard, 2007). While the Americans and the English also conducted research during WWII, it was not until after the Allies learned of the German research into nerve agents that they took extensive interest in the development and production of this new class of chemical weapons (Szinicz, 2005).

In the early 1950's, a subsidiary of the Imperial Chemical Industry in Britain and Bayer in Germany both independently discovered VX during studies of potential insecticides (Coleman, 2005; Marrs, 2007). The G-series agents which preceded VX were more volatile and, as such, evaporated much more quickly. This greater persistence along with a higher acute toxicity are the primary factors why VX is considered the most effective chemical warfare agent ever produced. The United States produced GB at the Rocky Mountain Arsenal, CO and produced VX at the Newport Army Ammunition Plant in Indiana from 1961 to 1968 (MacNaughton & Brewer, 1994).

Chemical warfare agents have not been used on a large industrial scale since WWI. However, smaller regional conflicts have seen or had alleged usage of chemical warfare agents. Egypt reportedly used tear and mustard gas and possibly nerve gas against the Yemen Royalists during the Yemen Civil War (1963 – 1967) (Salem, Ternay Jr., & Smart, 2008). Allegations have been made that the Soviets used nerve agents, phosgene and tear gas in Afghanistan (1979 – 1989) (Salem, Ternay Jr., & Smart, 2008; Maynard, 2007). In the Iran-Iraq War (1980 – 1988), there was extensive use of mustard and probably nerve gas. Initially, the attacks were conducted as an Iraqi defensive tactic in response to Iranian human wave attacks in November 1980. In one attack, 5,000 Iranians and Kurds were reported to have died, which was the highest single combat related death toll from chemical weapons since WWI (Maynard, 2007). It was also reported that Libya used mustard agent against Chad in 1987 (Salem, Ternay Jr., & Smart, 2008).

In addition to the direct usage of chemical weapons, the testing and storage associated with their presence in a country's military stockpile presents a risk to the country's population. While the United States has never used nerve agents on the

battlefield, nor have they been used against the United States civilian population, two non-hostile incidents greatly affected the United States chemical weapons program. The first at Dugway Proving Ground in Utah involved leakage from an aerial spray tank, which was supposedly empty. The resulting contamination migrated over the borders of the installation into Skull Valley. As a result, 6,000 sheep were sickened or killed (Salem, Ternay Jr., & Smart, 2008). The second incident, on the island of Okinawa in 1966, was the result of Sarin (GB) leaking from a navel bomb that was part of a secret stockpile that was kept on the island. The leak resulted in injuries to 23 soldiers and 1 civilian. These two incidents directly contributed to President Nixon halting all United States' production of chemical weapons in 1969 (Salem, Ternay Jr., & Smart, 2008).

The chemical weapons convention that went into effect in 1997 called for halting programs currently developing chemical warfare agents and the destruction of all existing stockpiles. While the United States had already stopped production in 1969, it ratified the chemical weapons convention treaty and as of 18 April 2014, destroyed 28.8 K tons of its original supply of 31.5 K tons of chemical weapons, which included mustard agents, sarin, and VX (Centers for Disease Control and Prevention, 2014; Osborn, 2010). Current schedules have the stockpile in Pueblo, CO scheduled for destruction by 2017 and the stockpile in Blue Grass, KY scheduled for destruction by 2021. Even with the destruction of known stockpiles and international prohibitions on the future development and usage of chemical weapons, it is difficult to prevent rogue nations and terrorists from obtaining and using chemical weapons (Talmage, Munro, Watson, King, & Hauschild, 2007). The relative simplicity of production, the low cost, stability, and potency when

compared to both biological and nuclear weapons ensures chemical weapons will continue to maintain an appeal to both rogue nations and terrorist organizations.

The relative ease and low cost involved in manufacturing chemical weapons was shown in 1994 and again in 1995 when Aum Shinrikyo, “Supreme Truth”, conducted sarin gas attacks in Japan. The first attack targeted three judges and was conducted outside their apartment complex in the town of Matsumoto. Seven people were killed and 600, including the three judges, were injured or showed symptoms typically associated with nerve agent exposure (Maynard, 2007; Vale, Rice, & Marrs, 2007). The second attack targeted Tokyo subway stations that served governmental agencies. The cult members punctured plastic bags containing sarin, which is liquid at temperate temperatures, with the tips of umbrellas. In total, 5 subway cars were targeted on three different subway lines causing 5,000 people to report to medical facilities as casualties. Of those 5,000 people, 984 experienced moderate nerve agent poisoning, 54 experienced severe nerve agent poisoning and 12 people were killed (Vale, Rice, & Marrs, 2007). The severity of the attack was limited, however, because the sarin used was only 30% pure. Had additional effort been made to increase the purity, the number of people killed and contaminated would have been much higher (Talmage, Munro, Watson, King, & Hauschild, 2007).

Additional acts of terrorism have taken place with the use of chemical warfare agents. In 1982, in Chicago, Tylenol capsules were laced with 95-100 mg of cyanide, with the lethal dose being approximately 70 mg for an adult. This product tampering style terror attack was copied in 1986 with Lipton Cup of Soup, Excedrin, and Tylenol. In 1991, Sudafed was tampered with and in 1992 Goodies Headache Powder was also

tampered with (Salem, Ternay Jr., & Smart, 2008). In addition to the product tampering, it was reported that sodium cyanide was included as part of the bomb that was used in the first world trade center attack in 1993. Speculation exists that had the cyanide existed and aerosolized, it could have killed everyone in the north tower (Salem, Ternay Jr., & Smart, 2008).

Efforts to restrict access to chemical weapons and limit people's ability to use them is made difficult by the beneficial commercial uses of many chemical warfare agent precursor chemicals. Precursor chemicals for tabun (GA) can be found in petrol additives, hydraulic fluids, insecticides, flame-retardants, pharmaceuticals, detergents, pesticides, missile fuels, chemicals for the vulcanization of rubber, and chemicals used for the extraction of gold and silver ores. Precursor chemicals for sarin (GB) can be found in flame-retardants, petrol additives, paint solvents, ceramics, and antiseptics. Precursor chemicals for soman (GD) can be found in lubricants, cleaning and disinfectant products used in breweries, dairy processing and various other food processing equipment. Precursor chemicals for VX can be found in pyrotechnics, lubricant oil, insecticides and chemicals used for organic synthesis (Coleman, 2005). Efforts can be made to track the purchase and shipment of key precursor chemicals. However, the wide range of legal applications for chemicals capable of aiding in the synthesis of chemical warfare agents allows for relatively easy acquisition of the supplies needed to create a variety of chemical warfare agents.

Recently, exposure to sarin or sulfur mustard agents has been reported by military personnel serving in Iraq. At least 25 service members have come forward as part of a New York Times investigation claiming exposure to chemical warfare agents. A recent

review of Army Post Deployment Health Assessment Surveys indicates that 629 people answered, “yes” when asked if they thought they were exposed to chemical, biological, or nuclear warfare agents (Chivers, 2014). The sources of individual exposure varies but the primary exposure mechanism appears to be an improvised explosive devices either made from an old chemical weapons shell that still contained small amounts of agent, or an improvised explosive device purposefully filled with a chemical agent.

With the current state of conflict within the Middle East and Africa, the availability of chemical weapons production knowledge from countries who formerly possessed a chemical weapons program could be utilized by terrorist organizations. In 2002, an FBI bulletin indicated that al-Qaeda was trying to gain access to United States water supplies and waste water treatment plants. The FBI report was based on documents found in Afghanistan, including maps of United States municipal public drinking water systems and an intelligence report that indicated terrorists trained in Afghanistan were planning on attacking the United States water supply with cyanide (Salem, Whalley, Wick, Gargan II, & Burrows, 2008). Additionally, the National Research Council of the National Academy of Sciences reported that the disruption and contamination of the nation’s water supply should be considered as a possible terrorist objective (National Research Council, 2004).

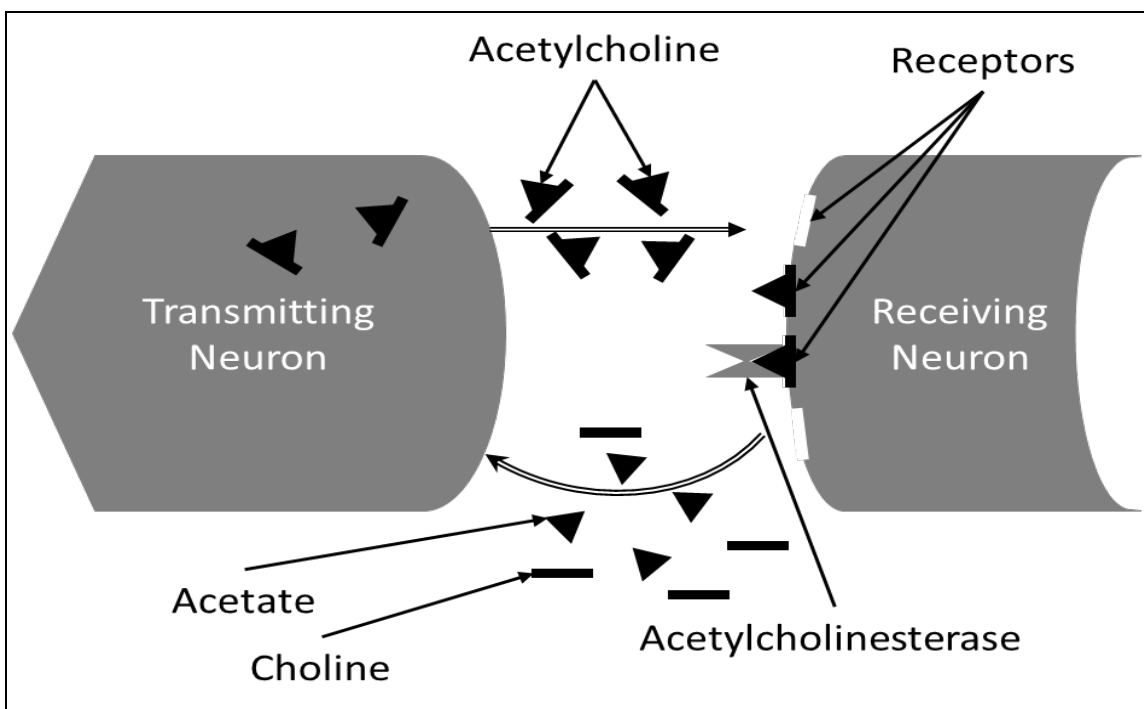
This is not the first targeting of an adversary’s water supply. In 600 B.C.E., the Assyrians poisoned wells, in 430 B.C.E. the Spartans were accused of poisoning water supplies with the plague, and in the American Civil War, Confederate troops, during their retreat from Vicksburg, contaminated both surface and subsurface water sources (Salem, Whalley, Wick, Gargan II, & Burrows, 2008). As a community’s water supply is vital to



not only human health, but is also integrated into manufacturing, power production, transportation, and many other facets of industry (McDonough & Romano Jr., 2008). It is important that we better understand the effects that chemical weapons would have, were they to be introduced into the water supply.

### **Nerve Agent Chemical and Toxic Properties**

Nerve agents are considered one of the most deadly chemical weapons, falling into the class of chemical compounds known as organophosphates, which makes nerve agents chemically related to insecticides such as parathion and malathion (Romano Jr., McDonough, Sheridan, & Sidell, 2001). The toxicity of organophosphates is derived from their ability to cause inhibition of acetylcholinesterase (AChE), which is responsible for breaking down the neurotransmitter acetylcholine (ACh). The accumulation of acetylcholine in the synaptic space between the transmitting and receiving neuron causes an overload to the receptors resulting in the degradation of both voluntary and involuntary nervous system functions (MacNaughton & Brewer, 1994; Waymire, 1997). Under normal conditions, acetylcholine is hydrolyzed by acetylcholinesterase into acetate and choline; this is illustrated in Figure 1.



**Figure 1: Acetylcholinesterase Hydrolysis Process**

There are two types of cholinergic receptors with which acetylcholinesterase is associated. Nicotinic receptors are located in the skeletal neuromuscular junctions, the sympathetic and parasympathetic nervous system, the autonomic ganglia, and the central nervous system. Muscarinic receptors are located in the parasympathetic nervous system, the sympathetic nervous system, and the central nervous system (CDC, 2012). If overstimulation of these receptors occurs, a variety of symptoms can be produced depending on the system impacted. For low dose exposures, expected symptoms are running nose, contraction of the pupils, blurred vision, slurred speech, nausea, and hallucinations (Coleman, 2005). For high dose exposures, expected symptoms are difficulty breathing, convulsions, deep coma, and death. For very high dose exposures, the symptoms are the same as for low and high dose exposures, however, symptoms

onset rapidly, with victims dying of suffocation as the respiratory and nervous systems fail at the same time (Coleman, 2005).

Treatment for exposure to nerve agents consists of three drugs: atropine, pralidoxime chloride (2-PAM Cl), and diazepam. Atropine blocks the effects of excess ACh at muscarinic receptor sites relieving the effects of further ACh stimulation. However, atropine has little effect on the nicotinic sites that control skeletal muscles. 2-PAM Cl is an oxime that breaks the organophosphate enzyme bond allowing for the reactivation of AChE inhibited sites, restoring normal strength and muscle skeletal control. Currently, 2-PAM Cl is the oxime of choice for the U.S. military (Moore & Alexander, 2001). Diazepam is an anticonvulsant drug and, unlike atropine and 2-PAM Cl, which are standard antidotal therapy drugs for most nerve agent poisonings, diazepam is only administered when severe nerve agent poisoning is observed. Diazepam is used to prevent brain damage associated with seizures that can result from severe nerve agent poisoning. When convulsions are not present but other symptoms of severe nerve agent poisoning are present, diazepam is still administered (Moore & Alexander, 2001).

Table 1 outlines key characteristics of each of the four most prevalent nerve agents along with malathion. While all four common nerve agents are highly toxic for their individual ability to inhibit ACh, VX is significantly more lethal than GA, GB, GD. The lethality stems from three different areas. First, its high LD<sub>50</sub>, that is the dosage expected to cause death in 50% of an exposed population. As indicated Table 1, the LD<sub>50</sub> for VX is between 0.043 – 0.143 mg kg<sup>-1</sup> that is equivalent to a 3 to 10 mg dose for a 70 kg adult. This is 34 times lower than the closest G agent, soman, which has an LD<sub>50</sub> of 5 mg kg<sup>-1</sup>. Second, VX is extremely persistent in the environment. The high persistence

is due to its very low volatility of  $10.5 \text{ mg m}^{-3}$ . This makes it the least likely of the four nerve agents to transition from a liquid to a gaseous state. The third component that makes VX so dangerous is its stability in water. At a neutral pH of 7, VX has a hydrolysis half-life of 1,000 hrs, which is 22 times greater than its closest counterpart, GD. The combination of these three properties is the driving factor behind this research. The long-term presence of VX in water, at a neutral pH, presents significant concern for any water or wastewater treatment system that is operating in the vicinity of any potential VX contamination.

**Table 1: Identity, Chemical and Physical Properties of Nerve Agents and Malathion**  
(Munro, et al., 1999; NPIC, 2009; ATSDR, 2003; MacNaughton & Brewer, 1994; Kikilo, Fedorenko, & Ternay Jr., 2008; CDC, 1994; Bartelt-Hunt, Knappe, & Barlaz, 2008; National Research Council, 1997)

	Chemical				
Property	GA (Tabun)	GB(Sarin)	GD(Soman)	VX	Malathion
Chemical formula	C <sub>5</sub> H <sub>11</sub> N <sub>2</sub> O <sub>2</sub> P	C <sub>4</sub> H <sub>10</sub> FO <sub>2</sub> P	C <sub>7</sub> H <sub>10</sub> FO <sub>2</sub> P	C <sub>11</sub> H <sub>26</sub> NO <sub>2</sub> PS	C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub>
CAS #	77-81-6	107-44-8	96-64-0	50782-69-9	121-75-5
Molecular Weight	163.1	140.1	182.2	267.4	330.36
Physical State	Oily Liquid	Liquid	Liquid	Oily Liquid	Liquid
Color	Colorless to Brown	Colorless	Colorless	Light amber/amber	Deep brown to yellow
Odor	Fruity	N/A	Fruity	N/A	Garlic
Melting point (°C)	-50	-57	-42	-39	2.9
Boiling Point (°C)	220-246	158	198	298	156-157
Density, liquid (g/ml)	1.0733 (25 °C)	1.102 (20 °C)	1.022 (25 °C)	1.008 (20 °C)	1.23 (25 °C)
Vapor Pressure (mmHg)	0.037 (20 °C)	2.10 (20 °C)	0.27 (20 °C)	0.0007 @ 20 °C	1.78 (25 °C)
Volatility (mg/m <sup>3</sup> )	610	2200	3900	10.5	*
Vapor density (air =1)	5.6	4.9	6.3	9.2	11.4
Water solubility (mg/L)	98000 (25 °C)	Miscible	21000 (20 °C)	30000 (25 °C)	145 (20 °C)
Hydrolysis rate (half-life)	8.5 hr (pH 7)	39 hr (pH 7)	45 hr (pH 6.6)	1000 hr (pH 7)	100 hrs (pH 7)
Henry's Law Constant	1.52 x 10 <sup>-7</sup>	5.4 x 10 <sup>-7</sup>	4.6 x 10 <sup>-6</sup>	3.5 x 10 <sup>-9</sup>	4.9 x 10 <sup>-9</sup>
Log Kow	0.384	0.299	1.824	2.09	2.36
Log Koc	2.02	1.77	1.17	2.5	3.25
LD <sub>50</sub> (mg/kg)	21	28	5	0.043 - 0.143	246 - 471

\* Not available

It is important to note differences between nerve agents and pesticides. Organophosphates, both nerve agents and pesticides, have similar anticholinesterase inhibitory capabilities and as a result, the general medical treatment strategies are similar (Marrs, 2007). While both cause cholinergic crisis as a result of acute intoxication, the effects of nerve agent intoxication, while more severe, are generally experienced over a much shorter time frame than pesticide intoxication. Additionally, pesticides have

exhibited the ability to produce both a delayed peripheral neuropathy and manifestation of organophosphate poisoning that is not typically seen in nerve agent poisoning (Benschop & De Jong, 2001).

Due to its highly toxic nature, the use of VX outside of nonsurety laboratories is restricted. This necessitates the use of a surrogate compound for research within our laboratory. Ideally, a VX surrogate would have similar physical and chemical properties that would allow for similar interactions within the environment of interest, while limiting or eliminating the risk of human toxicity (Bartelt-Hunt, Knappe, & Barlaz, 2008). Commonly used VX surrogates are Amiton (VG), O, S-diethyl phenylphosphonothioate (DEEP), malathion and parathion (Bartelt-Hunt, Knappe, & Barlaz, 2008). In the selection of a VX surrogate, the intended use is a critical factor as a given surrogate may only closely represent the physical or chemical properties of the agent of interest within only a single type of experiment. The following are areas that should be closely examined when choosing a surrogate chemical: sorption and desorption properties, volatility, biodegradability, hydrolysis, and toxicity (Bartelt-Hunt, Knappe, & Barlaz, 2008).

The rate of sorption and desorption from organic carbon is governed by the octanol water partition coefficient ( $\log K_{ow}$ ). VX and malathion have  $\log K_{ow}$  values of 2.09 and 2.36, respectively, indicating similar sorption and desorption properties (Bartelt-Hunt, Knappe, & Barlaz, 2008). Volatility, the likelihood that a substance will vaporize, is governed by constants derived from Henry's Law. The Henry's Law constant for VX is  $3.5 \times 10^{-9}$  and a value for malathion is  $4.9 \times 10^{-9}$ . As both numbers are on the same order of magnitude, they both should exhibit similar distribution between a liquid and

gaseous state (Bartelt-Hunt, Knappe, & Barlaz, 2008). Biodegradation is significantly affected by the compound of interest's chemical structure. It can also be affected by the compounds solubility in water. While all potential surrogates are significantly different in their chemical structurally, when compared to VX, malathion and amiton (VG) provide the closest representation. Hydrolysis, like biodegradation, is dependent on the chemical structure. In order for a surrogate to demonstrate similar hydrolysis rate reactions, the chemical bounds that are responsible for hydrolysis within the target compound must be present or be very similar to chemical bounds within the surrogate (Bartelt-Hunt, Knappe, & Barlaz, 2008).

As mentioned above, structurally, malathion and VX are different, however, under alkaline conditions, malathions hydrolysis rate is reasonably similar to VX (Bartelt-Hunt, Knappe, & Barlaz, 2008). While other surrogates, such as amiton, may provide a better representation of VX, its current classification as a schedule 2 substance and its higher mammalian toxicity make it a less than ideal choice. The selection of malathion provides the surrogate for VX with the lowest possible human toxicity while providing a compound that is impacted similarly by hydrolysis, biodegradation, and sorption/desorption (Bartelt-Hunt, Knappe, & Barlaz, 2008).

In addition to its physical and chemical similarities, the use of malathion has the additional benefit of providing insight into its own reactions within a waste water treatment plant. Malathion is used in insect eradication programs as well as on food crops, home mosquito control, and to medically treat head lice (Cox, 2003). The EPA estimates that the annual usage of malathion nationwide is over 30 million pounds, making it the most used insecticide in the United States (Cox, 2003). The results of any

test using malathion as a VX surrogate can be directly applied to any potential situation involving malathion contamination within a waste water treatment facility.

### **Low Level Exposure**

A low dose exposure to chemical warfare agents is defined in terms of exposure to levels that can be described as asymptomatic: they do not induce overt acute signs or symptoms (Scott, 2007). The primary concern of most research with regards to exposure to any nerve agent is focused on a large single dose due the agent's extraordinarily high acute toxicity (Romano Jr., McDonough, Sheridan, & Sidell, 2001). While studies have been done looking at both acute low dose and chronic repeated subclinical exposures to organophosphates, there is no common consensus among the results (Benschop & De Jong, 2001; Somani & Husain, 2001). However, studies have indicated that long term OP pesticide exposure in greenhouse workers may be associated with subtle adverse behavioral effects. The effects can be characterized by increased tension and anxiety, depression, fatigue, and a reduction of perceptual motor functions (Benschop & De Jong, 2001). The importance of better understanding the effects of short and long term low dose exposures is important for the development of safe standards for water quality with regards to nerve agent contamination. Currently, the Tri-Service Standards set acceptable exposure limits to military personnel. However, they are not considered acceptable for civilian populations or applicable to water quality standards (Salem, Whalley, Wick, Gargan II, & Burrows, 2008).

Human testing of VX has been limited. Nevertheless, testing on 54 subjects was conducted to examine the incipient toxicity, which occurs when erythrocyte



cholinesterase activity is inhibited 70% or more, as a result of being exposed to various types of drinking water that had been contaminated with VX. (Sidell, 2007). These tests indicated that the quality of water with which the VX was consumed greatly affected the time it took to experience incipient toxicity. Outward symptoms of VX ingestion are colicky pain, nausea, vomiting, diarrhea, and involuntary defecation (Sidell, 2007).

Table 2 shows various lengths of time at which ingesting of VX, in different sources of water, can cause incipient toxicity.

**Table 2: Incipient Toxicity from VX ingestion  
(Sidell, 2007)**

<b>Type of Water</b>	<b>Dose</b>	<b>Time until Effect</b>
Distilled water	400µg per 70 kg	1 day - fell to 22% of control
Distilled water with Tetra glycine hydroperiodide	400µg per 70 kg	16 hrs - fell to 17% of Control
Water treated with standard field kit	400µg per 70 kg	Day 4, in 4 or 8 subjects
Tap water	400µg per 70 kg	Day 4, in 6 or 9 subjects

### **Nerve Agent Decontamination**

The presence of chemical warfare agents is difficult to detect when active detection instrumentation is not used. Outside of a terrorist organization announcing their use or a known spill or leak, the first signs of the presence of a chemical weapon will most likely be the signs and symptoms of the personnel exposed (Moore & Alexander, 2001). If a chemical incident were to take place, hazardous material (HAZMAT) response teams would most likely be the ones responsible for initial interaction and decontamination of civilian casualties. These teams are typically located within existing fire departments and are equipped and trained to deal with accidental and intentional release of toxic chemicals. HAZMAT teams are well prepared and equipped

to facilitate decontamination of the team following an incident response. However, they are not capable of providing that same level of decontamination to a large population (Moore & Alexander, 2001).

The three primary ways of decontamination of large populations depends on the hazard type. For a vapor hazard, simply removing victims from the area may be significant enough to prevent further intoxication. For a contact hazard, visible droplets may be wiped or blotted off of equipment and personnel utilizing chemical absorption. If chemical absorbents are not available, personnel and equipment may be flushed with copious amounts of water (Moore & Alexander, 2001; Vale, Rice, & Marrs, 2007). In order to facilitate large scale decontamination, many emergency response plans call for the use of water as it is readily available to first responders at an incident site.

Studies have examined the three primary decontamination methods utilizing water alone, water with soap, and water with sodium hypochlorite solution (bleach). The use of water and soap provides slight improvement over water alone in the decontamination process. While beneficial, the use of soap creates a logistical problem for response personnel. The emergency responders would have to either keep an adequate supply of soap in their vehicles, which could limit space for other supplies, or they would have to find a local source to procure the soap. Both of these options would in some way limit the overall effectiveness of the responders. The use of bleach along with water is by far the most effective as both GB and GD are rapidly hydrolyzed at high pH levels (Vale, Rice, & Marrs, 2007). However, like the use of soap, the logistics of ensuring adequate quantities of bleach combined with the time it would take to mix the appropriate solutions would limit its effectiveness in a mass casualty situation (Moore & Alexander, 2001).

While the addition of soap and or bleach may produce a greater level of decontamination and potentially degradation of the chemical warfare agent, the use of water alone is very effective. The shearing force and dilution effects achieved while using a high volume of water at around 60 psi is sufficient to provide decontamination for large populations (Moore & Alexander, 2001).

The basis of civilian decontamination is the multistage showering of patients with as little clothing as possible (Roberts & Maynard, 2007). While ideal, logistically this may not always be possible. The situation and location of an incident will play a significant factor in what land and facilities are immediately available for decontamination efforts. Expedient decontamination with copious amounts of water, as already discussed, by the use of a fire hoses directly, is a possibility if other facilities are not immediately available. If time and facilities permit, makeshift showers may be created by utilizing the fire suppression system within a parking garage or other outdoor structure that allows for adequate air flow, ensuring that vapors do not linger in the decontamination area (Roberts & Maynard, 2007).

A potential negative consequence with using large amounts of water for decontamination efforts is that it generates a large quantity of contaminated wastewater that must be treated. Depending on the severity of the incident, it is possible that the water contamination could reach a concentration capable of producing casualties. This risk requires the close monitoring and treatment of water used for decontamination in order to limit the overall impact of the incident (Moore & Alexander, 2001). Wastewater may be collected inside a designated containment area protected by inflatable bunds or other impermeable barriers (Roberts & Maynard, 2007). If the wastewater is not

collected on site, depending on the type of drainage system being utilized, the contaminated wastewater could either be sent directly to local waterways via the storm water system or it could be sent to the local wastewater treatment facility via the sewage system. In either case, local utility companies should be informed so that they can take appropriate action (Roberts & Maynard, 2007).

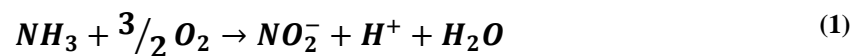
### **Wastewater Treatment Facility Operations**

Most wastewater treatment is either done in a private septic system or in a larger scale wastewater treatment system designed to support a community's needs. While direct research concerning the degradation of VX in a wastewater treatment facility is very limited, work can be found looking into other organophosphate chemicals, such as pesticides, as well as VX hydrolysis products, and their interactions within a wastewater treatment facility.

Each individual wastewater treatment facility is designed for specific parameters dealing with both the physical aspects of the facility as well as anticipated typical influent concentrations. The general process, however, for all plants is essentially the same. Municipal wastewater treatment systems generally begin with a preliminary treatment phase, which consists of bar screens or racks which are designed to physically remove coarse, non biodegradable debris (Droste, 1997). The primary treatment process removes large organic partials that have passed through the preliminary treatment. The wastewater is allowed to slowly pass through a clarifier which allows particles with a higher density than water to settle to the bottom and particles less dense than water, such as grease, to float to the surface. The solids are removed from the bottom and skimmed

off the top and are later processed along with the activated sludge. Removing a majority of the solids prior to the secondary treatment is more cost effective than aerobically treating all the waste in the secondary treatment phase (Droste, 1997).

The secondary treatment process consists of a series of aeration tanks and at least one if not multiple secondary clarifiers. Aerobic biological treatment takes place within the aeration tanks. Air is supplied to microorganisms which metabolizes organic material into CO<sub>2</sub> and various other end products. Activated sludge is a common aerobic biological treatment utilizing a suspended growth process. Air is pumped into the chamber to supply oxygen for microbial growth as well as ensuring that the chamber is thoroughly mixed. The activated sludge process can be designed to support nitrogen carbonaceous oxidation along with nitrification (Droste, 1997). Carbonaceous oxidation produces CO<sub>2</sub>, H<sub>2</sub>O, and NH<sub>3</sub> while nitrification, the oxidation of ammonia to nitrate, results in NO<sub>3</sub> and H<sub>2</sub>O. Nitrification occurs in a two-step process which is shown in equations (1) and (2).



Following the biological treatment within the aeration basin, aeration basin effluent is allowed to settle in the secondary clarifier to separate the water from the activated sludge. The activated sludge is then either recycled back into the aeration basin or thickened and further digested and dried before disposal in landfills or utilized in land

applications such as gardening and farming. The wastewater leaving the secondary clarifier then may go through a final treatment process to reduce any remaining pathogens within the water. The two primary sources of pathogen removal are either the addition of chlorine to the water or exposure to ultraviolet light (Droste, 1997). Following the disinfection process, the wastewater treatment process is considered complete and the water is discharged into a local waterway.

The amount of solid material that is removed from the system is driven by the facilities solid retention time, or the mean solids retention time. The sludge age, sludge retention time, and the mean solids retention time are all ways to determine the length of time, in days, the activated sludge stays within the system, shown in Equation 3 – Equation 5 (Kentucky Department for Environmental Protection, 2012). The sludge retention time is an important parameter within wastewater treatment plant design and an important factor for determining the types of microorganisms within the system. This is primarily driven by the amount of time a given type of microorganism needs to consume substrates within the wastewater and then reproduce. A sludge retention time set below the time needed for the microorganism to reproduce will result in the activated sludge being removed from the system faster than it can replenish itself (Droste, 1997). A sludge retention time that is set too high results in the activated sludge experiencing an environment with too little substrate, putting the organisms into a starved/stressed state. Sludge age can also have an impact on the food to microorganism ratio. If the food to microorganism ratio is not properly maintained, the settling rate can be affected, resulting in sludge making it past the secondary clarifiers and being discharged into the receiving body of water (Droste, 1997).

$$\text{Sludge age (days)} = \frac{\text{Total weight of MLSS in aeration basin}}{\text{Daily weight of TSS in the influent}} \quad (3)$$

$$\text{Sludge Retention Time (days)} = \frac{\frac{kg}{day} \text{ of suspended solids in aeration base}}{\frac{kg}{day} \text{ of suspended solids wasted from the system}} \quad (4)$$

$$\text{MCRT (days)} = \frac{kg \text{ MLSS in secondary system}}{\frac{kg}{day} \text{ of SS wasted} + \frac{kg}{day} \text{ of SS in the effluent}} \quad (5)$$

Where

MLSS = Mixed Liquor Suspended Solids

TSS = Total Suspended Solids

MCRT = Mean Cell Retention Time

A wide variety of bacteria can be present within a biological based treatment system, with each type of bacteria removing specific types of influent contamination and at times requiring specific growth conditions. Bacteria with specific growth requirements can be very susceptible to changes within their environment as well as the chemicals within the influent.

By mass, heterotrophic bacteria comprise the largest portion of the activated sludge and are the most tolerant to a variety of environmental changes. Heterotrophic bacteria are responsible for the removal of the majority of the organic content within the wastewater stream (Rittmann & McCarty, 2001, p. 309). The minimum sludge age to ensure adequate heterotrophic bacteria is 0.11 days. To account for variations in environmental conditions, the application of a safety factor of 36 dictates that facility design should allow for a sludge age of 4 days (Rittmann & McCarty, 2001, p. 309). Heterotrophic bacteria growth can be slowed or inhibited by a number of substances. Pesticides and antibiotic contamination are two primary chemicals that inhibit heterotrophic bacteria that can be found in domestic waste water. In addition to pesticides and antibiotics, heavy metals, aromatic hydrocarbons, and chlorinated solvents can be generated by industrial functions and can be found in the influent to a treatment facility. The presence of any of these substances can result in the inhibition of heterotrophic bacteria. (Rittmann & McCarty, 2001, p. 191).

In contrast to heterotrophic bacteria, which is fast growing and relatively tolerant to a variety of physical environments, nitrifying bacteria is slow growing and requires specific conditions in which to thrive. Nitrifying bacteria require significant amounts of oxygen to function; levels that heterotrophic bacteria could continue to thrive in would limit catabolic rate of nitrifying bacteria (Rittmann & McCarty, 2001, p. 471). In addition to the need for a high dissolved oxygen content, nitrifying bacteria is slow growing and sensitive to the sludge age maintained within the system. At 20 °C, the minimum sludge age is 1.5 days. Applying a safety factor of 5 gives a designed sludge age parameter of 18 days (Rittmann & McCarty, 2001, p. 473). The combination of a



slow growth rate in specific growing conditions makes nitrifying bacteria inhibition, potentially extremely detrimental to the treatment systems effective operation. Potential sources of nitrifying bacteria inhibition come from a variety of inorganic and organic compounds, such as unionized  $\text{NH}_3$ , undissociated  $\text{HNO}_2$ , anionic surfactants, heavy metals, and chlorinated organic chemicals. In addition to causing direct inhibition, some chemicals responsible for nitrification inhibition are also responsible for the reduction of dissolved oxygen concentrations further limiting nitrification (Rittmann & McCarty, 2001, p. 474).

### **Problem Statement**

The effect of nerve agents on human populations is relatively well understood. Numerous studies have looked into the toxicity and specific characteristics of a variety of nerve agents with respect to their effects on humans. Research is currently very limited in examining the specific effects of these compounds when introduced into a municipal wastewater treatment system. In the event of a nerve agent incident, it is possible that these chemicals may enter a wastewater treatment facility, either as a direct result of an attack, an accidental discharge, or as the result of a community's emergency response plan calling for the treatment of water used in decontamination efforts by the local municipality. If significant concentrations of organophosphates enter a biological treatment facility, the activated sludge responsible for the treatment of the water may be unable to simultaneously degrade the organophosphate while simultaneously producing effluent concentrations of Chemical Oxygen Demand (COD), Ammonia ( $\text{NH}_3$ ), Nitrate ( $\text{NO}_2$ ) and Nitrite ( $\text{NO}_3$ ) that meet the treatment facilities discharge permit requirements.

## **Research Objectives**

The purpose of this study was to determine experimentally the inhibition threshold of a municipal wastewater treatment facility's activated sludge when exposed to malathion, an organophosphate insecticide and surrogate for VX. Additionally, this study aimed to identify potential respiration rate indicators, the malathion degradation potential, and the effect on COD oxidation and nitrification process of activated sludge when exposed to various malathion concentrations.

## **Scope and Approach**

This research was designed to replicate the secondary treatment phase within a waste water treatment plant utilizing 2.0 L sequenced batch reactors. The initial batch reactor was seeded with activated sludge obtained from the Fairborn Water Reclamation Facility, Fairborn, OH. The initial reactor was allowed to stabilize for two months while being fed simulated wastewater designed to replicate the chemical structure typically found in the water being treated within a municipal wastewater facility. After the initial reactor stabilized, it was used to seed two additional 2.0 L batch reactors which in turn were allowed to operate until effluent testing indicated stabilization.

Respirometry experiments were conducted to examine the respiration rate of activated sludge over a twelve hour period when exposed to various concentrations of malathion. The respirometry results were then used to determine malathion concentrations for a series of bench scale batch tests looking at COD oxidation, nitrification, and malathion degradation. The concentrations used in the batch tests were then replicated within the 2.0 L batch reactors to examine the effects on effluent quality

of sustained long term exposure to malathion contaminated influent. This work builds upon experiments done to determine the ability of activated sludge to sorb and biodegrade malathion as well as ethyl methylphosphonic acid (EMPA) (Janeczko, et al., 2014). Malathion was selected as a surrogate for organophosphate based chemical weapons, nerve agents and specifically VX, due to similar chemical structure and mode of toxicity (Table 1). A significant difference, however, and one important to this research, is that malathion is much safer to handle than VX.

### **Significance**

In the event that a chemical warfare incident should occur, contamination has the potential to be widespread. Expedient mass causality decontamination efforts will most likely involve copious amounts of water that may be inadvertently or intentionally directed into a wastewater treatment facility. There is a risk that these chemical warfare agents may have an adverse impact on a wastewater treatment plant's ability to continue to operate at high enough efficiency to ensure effluent water qualities are within standards. Effluent discharge that has not been fully treated can pose significant risk to the environment downstream of plant discharge, which can translate into human health concerns for communities who use downstream bodies of water as sources of food, drinking water or other industrial purposes. It is important to understand the behavior of these organophosphate based compounds within an activated sludge treatment systems so that early warnings of system degradation can be detected and appropriate action taken to mitigate against low quality effluent discharge.

**Preview**

This thesis is written in the scholarly article format. Chapter 2 is a journal article produced with this research for future journal submission. It is written as a standalone chapter and includes an abstract, introduction, materials and methods, results, discussion, and conclusions. Chapter 3 provides final discussion of the conclusions presented in Chapter 2. It also summarizes pertinent findings, limitations identified within the research, and future research options not discussed in Chapter 2.

## II. Scholarly Article

### Abstract

Decontamination activities following a chemical warfare agent (CWA) incident may generate significant quantities of contaminated wash water. The disposal method identified for this water will be specific to each location, with some communities choosing to utilize the local wastewater treatment plant to process and the waste. This raises concerns about the effects of CWAs on treatment facilities, which utilize biological wastewater treatment methods. These facilities serve as an important line of defense against the spread of pollutants in the aquatic environment; however, the presence of CWAs in the influent stream may inhibit the microbial action that is responsible for remediating contaminated wastewater.

The goal of the current study was to evaluate the effect of malathion on the activity and performance of activated sludge bioreactors. Malathion is one of many organophosphate (OP) based pesticides and is considered a well-rounded surrogate for VX, an OP based CWA. This study employed respirometry, short term batch tests, and long term exposure experiments to investigate the effects of different concentrations of malathion on activated sludge performance.

Respirometry results showed that the maximum respiration rates were approximately  $45 \mu\text{g O}_2 \text{ min}^{-1}$  when the sludge was not exposed to malathion. However, when malathion was added over a range of concentrations between  $0.1 \mu\text{g L}^{-1}$  and  $5 \text{ mg L}^{-1}$ , the maximum respiration rates varied between 33 and  $59 \mu\text{g O}_2 \text{ min}^{-1}$ . The oxygen consumption curves were similar in each case, beginning with rapid oxygen

consumption for the initial 2.5 – 3 hours, followed by a gradual, nonlinear decline in the respiration rates until the experimental time reached approximately 5 hours when the respiration rates were generally between 5 – 15  $\mu\text{g O}_2 \text{ min}^{-1}$ .

Short term batch tests showed that chemical oxygen demand (COD) removal was not negatively impacted by the presence of malathion concentrations of 0.1 or 3  $\text{mg L}^{-1}$ . However, ammonia removal was slowed by the presence of malathion at both 0.1 and 3  $\text{mg L}^{-1}$  with a positive correlation of the removal rate, to the quantity of total suspended solids. Despite variations in the ammonia removal rate the final ammonia concentrations were unaffected by the presence of malathion at both 0.1 or 3  $\text{mg L}^{-1}$  when compared against the control. Long term exposure experiments demonstrated that both COD and ammonia removal were negatively affected at concentrations of 3.0  $\text{mg L}^{-1}$  and unaffected at concentrations of 0.1  $\text{mg L}^{-1}$ .

Short-term exposure to malathion is unlikely to interrupt microbial respiration, COD removal, or nitrification in the range of concentrations tested in this study. However, long-term exposure to malathion has the potential to negatively impact COD removal and nitrification processes at or above 3.0  $\text{mg L}^{-1}$ .

Key words: Organophosphate, malathion, activated sludge, respiration, performance degradation

## Introduction

Chemical weapons have been widely used throughout history dating back to around 2000 B.C.E. (Coleman, 2005). While chemical weapons have a long history, the beginning of industrial level chemical warfare began on 22 Apr 1915 when the Germans launched a chlorine gas attack against Allied positions at Ypres (Maynard, 2007; Spiers, 2010). Following World War I, during research intended to find a more effective pesticide; Dr. Gerhard Schrader of Germany isolated the first organophosphate based chemical warfare agent, tabun, later referred to as GA. Following this initial discovery of tabun, sarin (GB) was discovered in 1938, and soman (GD) was discovered in 1944 (Salem, Whalley, Wick, Gargan II, & Burrows, 2008), each discovery resulting in a more toxic compound. In 1952, *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX), considered to be the most toxic chemical warfare agent, was discovered. The discovery of VX happened simultaneously by a subsidiary of the Imperial Chemical Industry in Britain and Bayer in Germany, both attempting to develop more effective pesticides (Coleman, 2005; Marrs, 2007).

VX was chosen as the chemical warfare agent of interest, out of the four primary nerve agents, GA, GB, GD, and VX for three reasons: its toxicity, volatility and hydrolysis rate. VX is the most toxic of the 4 nerve agents with an LD<sub>50</sub> of 0.143 mg/kg or 10mg for a 70kg adult. This quantity is the dosage that is expected to cause death in 50% of an exposed population. Second, VX is the least likely to enter a vapor form as it has the lowest volatility with a vapor pressure of 10.5 mg/m<sup>3</sup>. Finally, the hydrolysis rate at a neutral pH is 1000 hours allowing VX to remain in its original form, within water, the longest of the 4 nerve agents.

While the chemical properties of each agent may differ, the lethal properties of all organophosphate compounds are primarily owed to their ability to irreversibly inhibit acetylcholinesterase, resulting in the breakdown of both the voluntary and involuntary nervous system functions (MacNaughton & Brewer, 1994). Symptoms of organophosphate exposure range from a runny nose and contraction of the pupils to breathing problems, convulsions and death from suffocation (Coleman, 2005). Low-level exposures are defined as exposure to levels that are asymptomatic in that they do not induce overt acute signs or symptoms. Low-level exposure can stem from inhalation or ingestion of, or contact with a chemical warfare agent. The concern over low level exposure stems from a lack of understanding of the effects of both acute low dose and chronic repeated subclinical exposures to organophosphates (Benschop & De Jong, 2001; Somani & Husain, 2001).

Due to the restrictions placed on live agent testing and the limitations of our laboratory, malathion was selected as safe surrogate for VX during our experimentation. Malathion closely resembles VX in four areas important in environmental degradation. The Log  $K_{ow}$  for VX and malathion are similar, 2.09 and 2.36 respectively, indicating that their affinity for sorption and desorption to organic carbon will be similar. Both VX and malathion's Henry's Law constant are on the same order of magnitude,  $3.5 \times 10^{-9}$  for VX and  $4.9 \times 10^{-9}$  for malathion, indicating that they both have a very low volatility. Additionally, malathion and VX share similar characteristics in their chemical structure, which allows malathion to mimic the biodegradation properties of VX. Furthermore, the similar chemical bonds also allow for malathion to have similar hydrolysis rates as VX, when operating under alkaline conditions. Of the potential VX surrogates, malathion



provides reasonable representation of VX in four key areas of environmental interaction while processing the lowest mammalian toxicity level (Bartelt-Hunt, Knappe, & Barlaz, 2008).

In the event of a chemical incident, a widely accepted option for first responders in decontamination of large populations is the use of large quantities of water. While each situation is unique, first responders have a variety of options for the use of water in the decontamination process. The use of fire hoses directly is a possibility if other facilities are not immediately available. Makeshift showers may be created by utilizing the fire suppression system within a parking garage or other outdoor structure, which allows for adequate ventilation (Roberts & Maynard, 2007). The resulting contaminated water may be diverted to a wastewater treatment facility for processing. If organophosphates are sent to an activated sludge treatment system, they may pass through the system without experiencing significant degradation depending on the compound and the influence of heterotrophic and nitrifying bacteria (Janeczko, et al., 2014). However, the threshold concentration of organophosphates in the influent, which results in decreased plant performance is largely unknown. If the treatment plant performance decreases, effluent concentrations of Chemical Oxygen Demand (COD), Ammonia ( $\text{NH}_3$ ), Nitrite ( $\text{NO}_2$ ), and Nitrate ( $\text{NO}_3$ ) could reach levels that negatively impact the local ecosystem, which is dependent on the discharge waterway.

The goal of this research was to examine the threshold concentration of malathion within a municipal waste water treatment systems, utilizing activated sludge, that would allow for continuous operation while functioning within its normal operating parameters. This was accomplished by utilizing bench scale tests, which focused on the activated

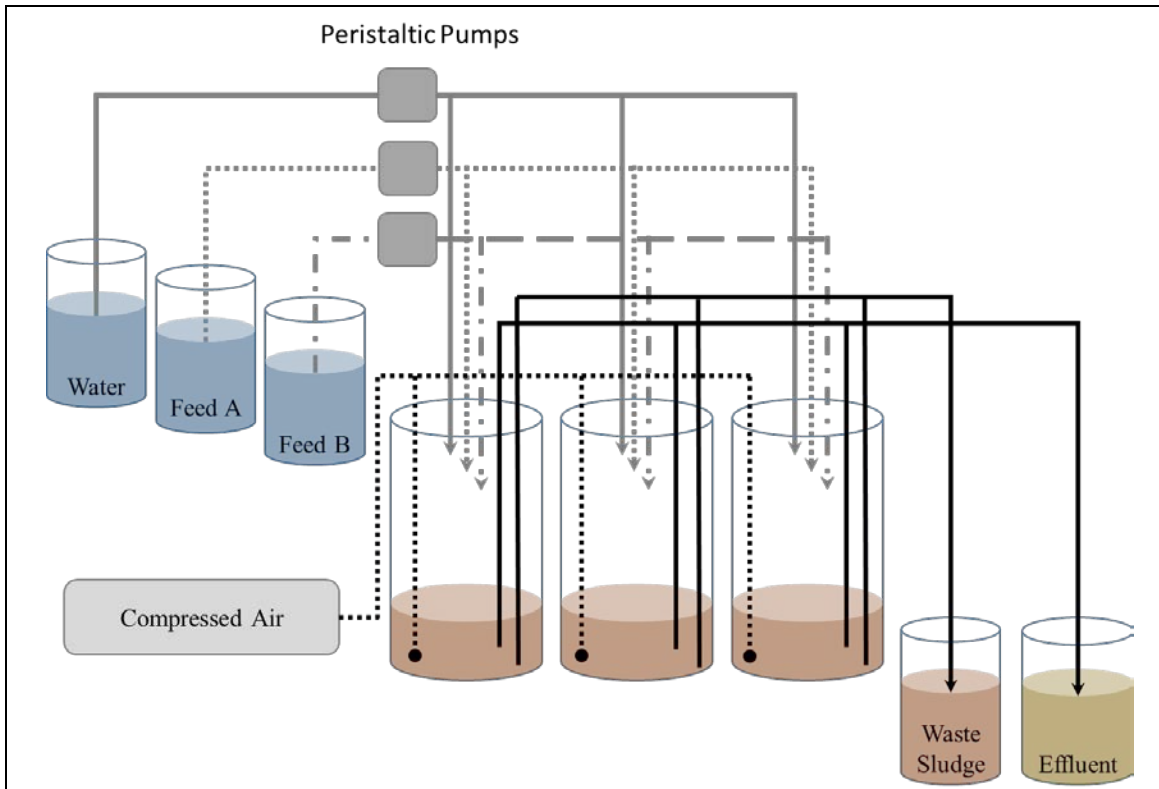
sludge's respiration rate and its ability to continue to perform COD oxidation and nitrification. Additionally, tests were performed to assess activated sludge's ability to degrade the malathion contamination.

## **Materials and Methods**

### ***Sequencing Batch Reactors***

Three 2.0 L sequencing batch reactors were constructed and tested for operation capability using water, ensuring accurate pump calibration and verifying computer controlled solenoids integrated into the pneumatic control system produced the desired results. The sequencing batch reactor configuration was developed utilizing a method adopted from Racz, et al. (2010) and is diagrammed in Figure 2. After the controls were verified, a single sequencing batch reactor was seeded with activated sludge from the Fairborn Water Reclamation Facility, Fairborn, OH, with 4 L of initial seed sludge collected 4 April 2014.

The Fairborn Water Reclamation Facility has a designed treatment volume of 6.0 million gallons per day. The daily plant flow rate is between 3.4 and 10 million gallons per day with an average of 4.2 million gallons per day. The Fairborn Water Reclamation Facility averages 98% removal of all suspended solids, biological oxygen demand, and ammonia as well as an average removal of 82% of all phosphorus (Fairborn Water Reclamation Center, 2010). The Fairborn Water Reclamation Facility maintained an average mean cell retention time of 13.68 days between January 2014 and November 2014 (Barosky, 2015).



**Figure 2: Sequence Batch Reactor Setup**

In order to replicate the waste water stream, two feed solutions were utilized. Each feed solution was made exclusively, utilizing deionized water, to prevent any interference from ions such as calcium, magnesium, or lead that are commonly found in tap water (Illinois Department of Public Health, 2014). Feed A consisted of 44.6 g sodium bicarbonate ( $\text{NaHCO}_3$ ) per liter of water. The sodium bicarbonate provided for a pH between 6.5 and 7.5, allowing for a relatively consistent alkalinity as well as acting as an inorganic carbon source during nitrification. Feed B acted as the waste water simulant and consisted of the following per liter of deionized water: 6.0 g of peptone, 1.25 g of sodium acetate ( $\text{C}_2\text{H}_3\text{NaO}_2$ ), 2.26 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ), 6.86 g of magnesium

chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ), 1.72 g calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), 0.6675 g potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) and 20 ml of a trace element solution.

The trace element solution contained the following per liter of deionized water: 2.73 g of citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ), 2.0 g of hippuric acid ( $\text{C}_9\text{H}_9\text{NO}_3$ ), 0.36g of trisodium nitrilotriacetate ( $\text{Na}_3\text{NTA} \cdot 2\text{H}_2\text{O}$ ), 0.15g of EDTA trisodium salt ( $\text{Na}_3\text{EDTA} \cdot 4\text{H}_2\text{O}$ ), 1.5 g of iron(III) chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), 0.25 g of boric acid ( $\text{H}_3\text{BO}_3$ ), 0.15 g of zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), 0.12 g manganese(II) chloride ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ), 0.07 g of copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), 0.03 g of potassium iodide (KI), 0.03 g of sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ), 0.03 g cobalt dichloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ), 0.03g of nickel chloride ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ), and 0.03 g of sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ). Additional information regarding the chemical feed can be found in Appendix A.

All three reactors operated continuously on identical 12-hour cycles consisting of three distinct phases. The first phase began with 1.33 L of activated sludge in each reactor. Then 624 ml of deionized water, 8 ml of Feed A, and 38 ml of Feed B was added to each reactor, bringing the total volume within each reactor up to its 2.0 L operating volume. Following this initial filling and feeding cycle, an 11-hour aeration cycle began. During this 11 hour cycle, the mixed liquor is aerated using laboratory compressed air that is passed through an M-26 motor guard submicronic compressed air filter utilizing an M-273 motor guard cellulose filter element and a pressure regulator. The compressed air ensures adequate mixing and sufficient dissolved oxygen concentrations within each reactor to support nitrification. During the aeration phase, each reactor was set to waste between 30 and 50 ml of mixed liquor keeping the solids retention time to an average of approximately 27 days. The solids wasting took place at

hour 6, halfway through the aeration cycle. The third phase began with a 1 hour settling period, ensuring all suspended biomass was able to settle to the bottom of the reactor. After the settling period, 670 ml of effluent was decanted from the top of the reactor, bringing the volume back down to the first phase starting volume of 1.33 L. This removal process results in a 36 hour hydraulic retention time.

During the initial months of testing, the reactors were continually monitored for COD,  $\text{NH}_3$ ,  $\text{NO}_2$ , and  $\text{NO}_3$  concentrations in the effluent. This period allowed for the development of long-term baseline data and for the activated sludge to stabilize in its new environment. Additionally, this period allowed for periodical removal of activated sludge for both the respirometry and bench scale batch test experiments to be carried out without creating a significant impact to overall reactor performance. Over the course of simultaneous reactor operations, 4 April 2014 to 9 Dec 2014, reactors 1, 2, and 3 facilitated the average reduction of chemical oxygen demand over the 12 hour reaction period from approximately  $116 \text{ mg L}^{-1}$  to  $14.61 \text{ mg L}^{-1}$  (87.29%),  $15.10 \text{ mg L}^{-1}$  (86.87%), and  $16.45 \text{ mg L}^{-1}$  (85.70%) respectively. Additionally, over this period, each reactor reduced the initial concentration of ammonia from approximately  $12.21 \text{ mg L}^{-1}$  down to below the  $0.4 \text{ mg L}^{-1}$  detection limit. Reactor 1 had an average suspended solids concentration of  $2800 \pm 648 \text{ mg L}^{-1}$  with volatile suspended solids comprising an average of 89.76% of the total suspended solids. Reactor 2 had an average total suspended solids concentration of  $2643 \pm 802 \text{ mg L}^{-1}$  with volatile suspended solids comprising an average of 94.53% of the total suspended solids. Reactor 3 had an average total suspended solids concentration of  $1971 \pm 365 \text{ mg L}^{-1}$  with volatile suspended solids comprising an average of 91.39% of the total suspended solids.

Following the respirometry and batch tests experiments, reactor 1 and reactor 3's supply of deionized water was substituted with an aqueous solution of malathion in deionized water at concentrations of  $0.3205 \text{ mg L}^{-1}$  and  $9.615 \text{ mg L}^{-1}$  respectively. The malathion concentrations supplied to each reactor, along with feed A and feed B volumes mentioned earlier, brought the concentration within reactor 1 to  $0.10 \text{ mg L}^{-1}$  and the concentration within reactor 3 to  $3.00 \text{ mg L}^{-1}$ . This design concentration was in addition to any malathion that may not have been degraded or removed through effluent or mixed liquor discharges. This substitution of the supply water allowed for the reactors to operate within the same mechanical parameters as they had previously while simulating a long term exposure to a malathion contaminated waste stream.

### ***Respirometry***

This experiment was conducted with 8 Pyrex - 250 ml - #1395 bottles. To replicate the environment within the sequence batch reactors, 275 ml of activated sludge was taken from reactor 1 and combined with 275 ml of solid purge waste sludge, also from reactor 1. This was done to decrease the impact to the bacterial community within reactor 1 while maintaining a consistent source for the activated sludge. Replicating the cycles within the sequencing batch reactors, 550 ml of activated sludge was allowed to settle for one hour in a 1000 ml beaker. After the one-hour settling period, 184.25 ml of effluent was decanted off the top of the beaker. The decanted volume was then partially replaced with 171.6 ml of deionized water. The activated sludge was then placed on a stir plate to completely suspend all solids. With the solids in suspension, 100 ml was distributed to two 250 ml bottles, bottles 1 and 2. The remaining six bottles each

received 50 ml of activated sludge, bottles 3 through 8. Feed A was then added to each of the 8 bottles, 400  $\mu\text{l}$  to bottles 1 and 2, and 200  $\mu\text{l}$  to bottles 3 - 8. Feed B was then added, 1900  $\mu\text{l}$  to bottles 1 and 2, and 950  $\mu\text{l}$  to bottles 3 - 8. Following the addition of the feed, 50 ml samples were taken from bottles 1 and 2 to establish the starting conditions for all eight vials. After the samples were filtered from jars 1 and 2, malathion was added to bottles 3 through 8 in quantities sufficient to reach the desired starting concentrations shown in Table 3.

**Table 3: Respirometry Malathion Concentrations**

Bottle	Test 1 ( $\mu\text{g L}^{-1}$ )	Test 2 ( $\mu\text{g L}^{-1}$ )	Test 3 ( $\mu\text{g L}^{-1}$ )	Test 4 ( $\mu\text{g L}^{-1}$ )	Test 5 ( $\mu\text{g L}^{-1}$ )	Test 6 ( $\mu\text{g L}^{-1}$ )
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	5.0	5.0	1.0	5,000	5,000	5,000
4	5.0	5.0	1.0	5,000	5,000	5,000
5	1.0	1.0	0.1	1,000	1,000	1,000
6	1.0	1.0	0.1	1,000	1,000	1,000
7	0.1	0.1	0.05	100	100	50
8	0.1	0.1	0.05	100	100	50

Immediately following the addition of malathion, the bottles were connected to a respirometer which measured the oxygen consumption and carbon dioxide production over a twelve hour period. The respirometer consisted of a Columbus Instruments micro-oxymax system sample pump, a sample drier, an oxygen sensor, a carbon dioxide sensor, and two 10-port expansion interface units. The samples were continually mixed utilizing an IKA 15 position stirring plate set to level 3, approximately 330 rpm. Sample frequency was set to auto, which equated to a 54 minute cycle between samples. The oxygen refresh threshold percentage was set 0.50. Unit parameters were selected as  $\mu\text{g}$

(gas), min (time), O<sub>2</sub> consumption was indicated as positive. The system was controlled utilizing the mciro-oxymax software V2.3.5 running on Windows 98.

### ***Inhibition and Biodegradation***

The short-term batch tests were conducted in three 1000 ml beakers. In preparation for the initial batch test, 1500 ml of sludge was taken from the reactors and mixed with 1500 ml of wasted sludge to provide sufficient volume for the batch test experiments while limiting the impact to ongoing reactor performance testing. Activated sludge source and quantities varied between batch tests due to ongoing experiments within the sequencing batch reactors. For specific information for each batch test see Table 4. Similar to the respirometry experiments, the sludge was mixed in a 4000 ml beaker and then allowed to settle for one hour. After the one hour settling period, 1005 ml of effluent was decanted off the top of the beaker. The decanted volume was then partially replaced with 936 ml of deionized water. Each beaker was then supplied with activated sludge, feed A, feed B and a 100 mg L<sup>-1</sup> solution of malathion in water to replace the remaining volume and generate the desired operating concentration. See Table 5 for specific volumes of each component. Every hour, over a twelve-hour period, concentrations of COD, NH<sub>3</sub>, and NO<sub>3</sub> were measured. Additionally, NO<sub>2</sub> was measured every 3 hours and gravimetric measurements were taken every 6 hours. These samples were taken from the liquid phase of the activated sludge in order to gauge the performance of the heterotrophic and nitrifying bacteria. Thereby examining both the bacteria's ability to process the simulated wastewater and degrade the malathion.



**Table 4: Batch Test Activated Sludge Source Volumes**

	SBR 1 Vol (ml)	SBR 1 Purge Vol (ml)	SBR 2 Vol (ml)	SBR 2 Purge Vol (ml)	SBR 3 Vol (ml)	SBR 3 Purge Vol (ml)
Batch Test 1	500	500	500	500	500	500
Batch Test 2	750	750	750	750	0	0
Batch Test 3	0	0	750	2250	0	0

**Table 5: Batch Test Set Up Volumes**

	Concentration (mg L <sup>-1</sup> )	Activated Sludge (ml)	Feed A (ml)	Feed B (ml)	100 mg L <sup>-1</sup> Malathion in H <sub>2</sub> O (ml)	Total (ml)
Reactor 1	0.00	977	4	19	0	1000
Reactor 2	0.10	976	4	19	1	1000
Reactor 3	3.00	947	4	19	30	1000

### ***Extraction and Measurement of Malathion***

Samples of effluent were extracted using a BD Luev-lok syringe and then passed through a Cole-Parmer syringe filter (SFCA membrane with a GF pre-filter and an opening size of 0.2  $\mu$ m) to remove any suspended solids and stop all biological degradation processes. The filtered sample was placed in a 2 ml crimp top amber auto sampler vial and stored at 5°C until they could be analyzed by gas chromatography-mass spectrometer (GC-MS) and quantified against calibration curves of know standards. All samples were taken in duplicate with the exception of the batch test samples, which were taken in triplicate.

### ***Gas Chromatography – Mass Spectrometer***

Malathion concentration samples were analyzed using an Agilent 7000C GC/MS Triple Quad, and a 7890B GC system utilizing a 7693 Auto Sampler, a 7693A Auto injector module with a 10  $\mu$ l syringe, an Agilent 5190-2293 inlet liner, and an Agilent

HP-5MS Ultra inert column (0.25  $\mu\text{m}$  film thickness, 0.250 mm internal diameter x 30 m length). Detection and quantification of malathion was accomplished in multiple reaction monitoring (MRM) for a more reliable identification of detected analytes and enhanced sensitivity (Schreiber, 2010). The column was held at an initial temperature of 45° C for 2.25 minutes. The temperature was then increased at a rate of 15° C/minute until it reached 260° C where it was held for 5 minutes. Post run, the temperature was increased to 265° C and held for 5 minute to reduce the possibility of carryover between samples. The column was supplied with helium (He) as the quench gas at a rate of 1.5 ml minute<sup>-1</sup> and nitrogen (N<sub>2</sub>) as the collision gas at 2.5 ml minute<sup>-1</sup>. The injection port inlet was kept at 175° C and a pressure of 7.3614 psi. The total flow rate in the inlet was 24 ml minute<sup>-1</sup> of He, the septum purge flow was 3 ml minute<sup>-1</sup>. The mass spectrometry transfer line was kept at 250° C and the triple quad MS solvent delay was set at 3.75 minute.

During detection, the peak width filter was set at 0.8 sec with a calculate EMV detector voltage of 1195.9 V with a gain of 10. Sampling was accomplished at 47 cycles per second with each cycle taking 21 ms. The precursor ion was selected as 173 and the product ion was selected as 99, both utilizing the widest resolution setting. The dwell was set to 20 and the collision was set to 10. The sample injection volume was set at 5  $\mu\text{l}$ . After sample injection, the syringe was rinsed two times in solvent A, methanol, and two times in solvent B, methanol. Prior to sample injection, the syringe was rinsed three times in solvent A, three times in solvent B, and then was rinsed using the sample solution three times. All syringe rinses were completed utilizing the max volume setting. Following the pre-injection rinse cycle, the sample solution was pumped in and out of the

syringe 6 times prior to sample injection. Utilizing total ion chromatography, malathion had a retention time of 15.5 minutes, with a total run time of approximately 32.2 minutes per sample; variations of total run time were primarily due to the effects of room temperature on system cooling between samples.

Calibration curves were developed utilizing nine concentrations between  $10 \text{ mg L}^{-1}$  and  $1 \text{ } \mu\text{g L}^{-1}$  of malathion in methanol as the initial portion of an analytical sequence. All calibration curve samples were generated using an Agilent 7696A Sample Prep WorkBench utilizing two 7693A Auto injector modules. The front 7693A was equipped with a 500  $\mu\text{l}$  syringe and the rear 7693A was equipped with a 100  $\mu\text{l}$  syringe. The WorkBench performed serial dilutions shown in Table 6 utilizing a  $1000 \text{ mg L}^{-1}$  malathion in methanol solution as the initial sample concentration. In addition to the calibration curve samples, multiple check samples were created with the WorkBench. Utilizing multiple check samples, all created from a single source sample, which was used to create the calibration curve, allowed for uncontaminated check samples to be used during each check sequences.

**Table 6: Calibration Curve Dilution Series**

Step	Target Concentration (mg L <sup>-1</sup> )	Volume - Starting Concentration	Methanol (μl)	Final Vol (μl )*
1	100	100 μl – 1000 mg L <sup>-1</sup>	900	1000 (900)
2	10	100 μl – 100 mg L <sup>-1</sup>	900	1000 (810)
3	5	50 μl – 100 mg L <sup>-1</sup>	950	1000
4	1	140 μl – 10 mg L <sup>-1</sup>	1260	1400 (650)
5	1	150 μl – 1 mg L <sup>-1</sup>	0	150
6	1	150 μl – 1 mg L <sup>-1</sup>	0	150
7	1	150 μl – 1 mg L <sup>-1</sup>	0	150
8	1	150 μl – 1 mg L <sup>-1</sup>	0	150
9	0.5	50 μl – 10 mg L <sup>-1</sup>	950	1000
10	0.1	100 μl – 1 mg L <sup>-1</sup>	900	1000 (850)
11	0.05	50 μl – 1 mg L <sup>-1</sup>	950	1000
12	0.01	100 μl – 0.1 mg L <sup>-1</sup>	900	1000 (900)
13	0.005	50 μl – 0.1 mg L <sup>-1</sup>	950	1000
14	0.001	100 μl – 0.01 mg L <sup>-1</sup>	900	1000
* Numbers in parentheses is volume remaining after use in subsequent steps				

### ***Other Analytical Methods***

Concentrations of Chemical Oxygen Demand (COD), Ammonia (NH<sub>3</sub>-N), Nitrate (NO<sub>3</sub>-N), and Nitrite (NO<sub>2</sub>-N) were measured using HACH methods 8000 (low range), 10031, 10020, and 8153. Total suspended solid (TSS) and volatile suspend solid (VSS) were measured using standard methods (APHA, AWWA, WPCF, 1985). All HACH measurements were conducted in triplicate; all TSS and VSS measurements were conducted in duplicate.

## **Results and Discussion**

### ***Effects of Malathion on Respiration***

The results showed that the maximum respiration rates were approximately 47 μg O<sub>2</sub> min<sup>-1</sup> when the activated sludge was not exposed to malathion. When malathion was added, the maximum respiration rates varied between 40 and 46 μg O<sub>2</sub> min<sup>-1</sup>. The shape

of the oxygen consumption curves were similar in all cases, beginning with a rapid oxygen consumption rate during first 1.5 to 2 hours, followed by a gradual nonlinear decline in the respiration rates until the experimental time reached approximately 6 hours. The respiration rates after hour 6 were between 5 and 15  $\mu\text{g O}_2 \text{ min}^{-1}$ . Cumulative  $\text{O}_2$  consumption varied between 7 and 20 grams of oxygen over the 12 hour experiment across all 6 trials, but it was typically smaller when malathion was present.

Figure 3 shows the cumulative oxygen consumed over a 12 hour period during respirometry experiment 6. When the initial concentrations of malathion were 5, 0.1, and 0.05  $\text{mg L}^{-1}$  the average cumulative oxygen consumptions were 12.8, 13.3, and 11.4 g of oxygen respectively. Carbon dioxide production profiles matched the trends shown by the oxygen consumption profiles. These results showed indications that respiration rates may be slightly reduced by the presence of malathion.

The previous two tests, experiment 4 and 5 with similar malathion concentrations in the low part per million produced similar results as experiment 6. In experiment 4 and 5, the two control samples are isolated at the top of the cumulative oxygen consumption curve and the remaining samples intermixed below the control samples with a clear delineation between the control sample and those exposed to malathion. The three experiments conducted in the low parts per billion range, experiments 1 through 3, had less delineation between the control and contaminated samples with multiple samples consuming greater quantities of oxygen than the control samples. The results at these low concentrations demonstrated that concentrations of malathion in the low parts per billion either has no effect or potentially generates a slight increase to respiration rates. The remaining five cumulative oxygen consumption graphs can be found in Appendix B.

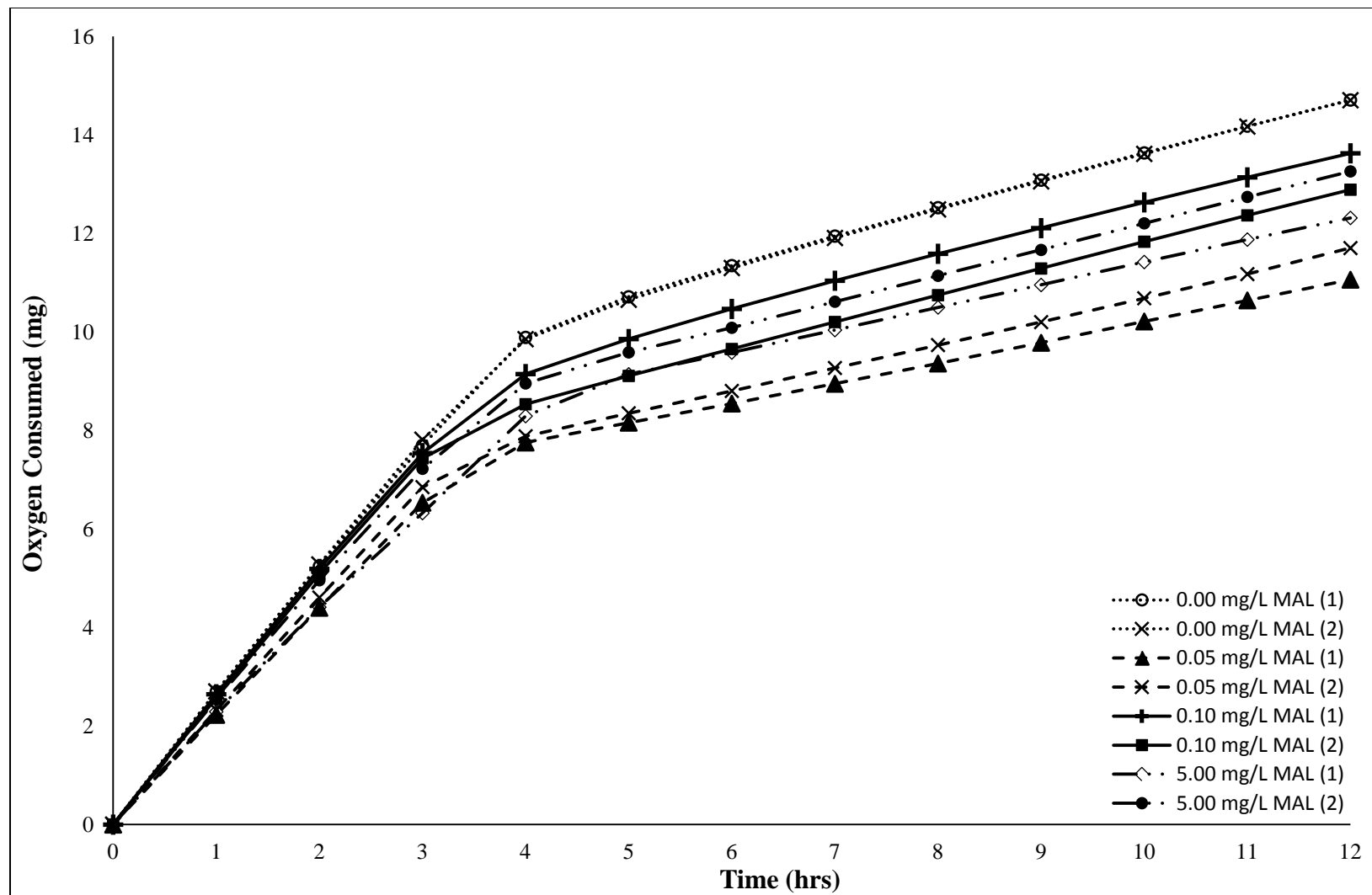


Figure 3: Respirometry Test 6 – Cumulative Oxygen Consumption

Figure 4 shows the mean respiration rate during the first hour, the first 6 hours, and the full 12 hours, measured in all six experiments utilizing Standardized Oxygen Uptake Rates (SOUR). The VSS concentrations utilized in generating this data was based off the percentage of VSS measured in reactor 1, the source of activated sludge for all 6 experiments, and applied to the TSS measurements taking during respirometry as only TSS data was calculated during respirometry experiments.

The normalization of the oxygen uptake across all 6 experiments shows that during the first 60 minutes, it was observed that the addition of malathion produced a decrease in the SOUR for all concentrations except  $5000 \mu\text{g L}^{-1}$  which had a measured increase of 2.3%. After 6 hours of exposure, a depressed respiration rate was observed in all samples when compared to the control with the exception of the  $50 \mu\text{g L}^{-1}$  which indicated a 9.2% increase in respiration. After 12 hours of exposure, the trend continued with all samples indicating respiration rates lower than the control with the exception of the  $50 \mu\text{g L}^{-1}$  sample which had a 1.6% increase in respiration rate over the control.

The SOUR results indicate variations of expected peak oxygen consumption based upon the level of malathion present. Low-level concentrations of 0.05 through  $50 \mu\text{g L}^{-1}$  of malathion had peak oxygen consumption rates between hours 2 and 6. Oxygen consumption rates above  $100 \mu\text{g L}^{-1}$  of malathion peaked during hour 1 and then slowly declined over the course of the 12 hour experiment. These results show indications that there is not a linear relationship between the concentration of malathion and the respiration inhibition for concentrations below  $5000 \mu\text{g L}^{-1}$ .

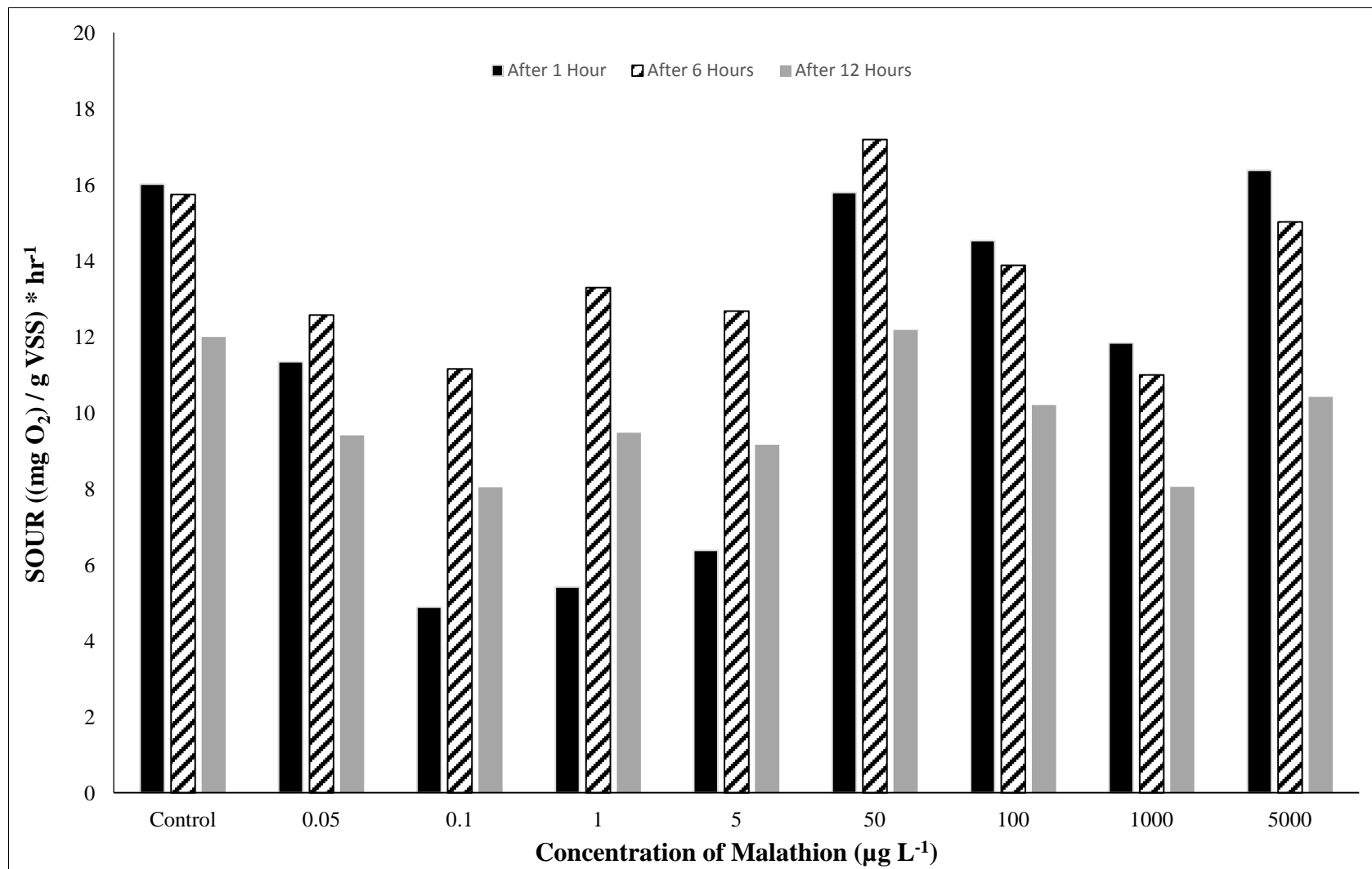


Figure 4: SOUR for Malathion Concentrations in Activated Sludge



Examination of percent inhibition provides additional insight into malathion's effect on activated sludge. Figure 5 shows the respiration inhibition plotted against malathion concentration. The percentage of inhibition plot shows two models. One model depicts the percentage of inhibition based upon the maximum respiration of the control sample against the maximum respiration rate within a sample exposed to malathion. The maximum respiration rates used to develop this inhibition rate all took place within the first 4 hours of the experiment. The inhibition percentage over the 12 hour period is based on the oxygen consumed during the duration of the experiment. The maximum inhibition percentage averaged 16%, 3%, and 11% for malathion concentrations of 50, 100, 5000  $\mu\text{g L}^{-1}$  respectively. The overall inhibition percentage averaged 26%, 13%, and 15% for the same concentrations. The linearization of these results shows that for both the maximum respiration rate and the overall respiration rate have a positive correlation between the concentrations of malathion and percentage of respiration inhibition, at a rate of 0.9% and 0.3% for each  $\text{mg L}^{-1}$  of malathion, respectively. However, the  $R^2$  values of 0.0766 and 0.0054 indicate that while the linearization of the data does show that there is an increase in the respiration inhibition as malathion concentration increases, there is only a very small correlation between the two variables.

These results are further supported by the previous 5 experiments which produced a variety of trend lines indicating both a positive and negative correlation between malathion with a wide range of  $R^2$  values, the highest being 0.4534 as shown in Table 7. The variations between the experiments 1 – 3 and 4 – 6 indicated drastically different rates of change in the inhibition percentage as the concentration of malathion changes.

Additionally, when examined based upon initial starting concentrations, neither the first 3 experiments in the low parts per billion nor the second 3 experiments in the low parts per million produced any significant correlation between the initial concentration of malathion and the amount of respiration inhibition that was experience; see Figure 6 and Figure 7. As a result of the vastly different outcomes between experiments, that data does not point to a concentration of malathion where a noticeable change in respiration is to be expected.

**Table 7: Rate of Percentage Inhibition Change due to Malathion Exposure**

	Maximum O <sub>2</sub> Uptake Rate		Average O <sub>2</sub> Uptake Rate	
Respirometry Test	Rate of Inhibition Change % (mg L <sup>-1</sup> ) <sup>-1</sup>	R <sup>2</sup>	Rate of Inhibition Change % (mg L <sup>-1</sup> ) <sup>-1</sup>	R <sup>2</sup>
1	- 1,050	0.0784	- 1,650	0.0784
2	- 1,470	0.0255	2,180	0.0642
3	3,790	0.0198	- 22,290	0.6952
4	- 1.0	0.2888	- 1.0	0.0714
5	3.0	0.4534	3.0	0.1946
6	0.9	0.0766	0.3	0.0054
1 – 3	-760	0.0097	2,000	0.0343
4 – 6	0.7	0.0286	0.6	0.0138

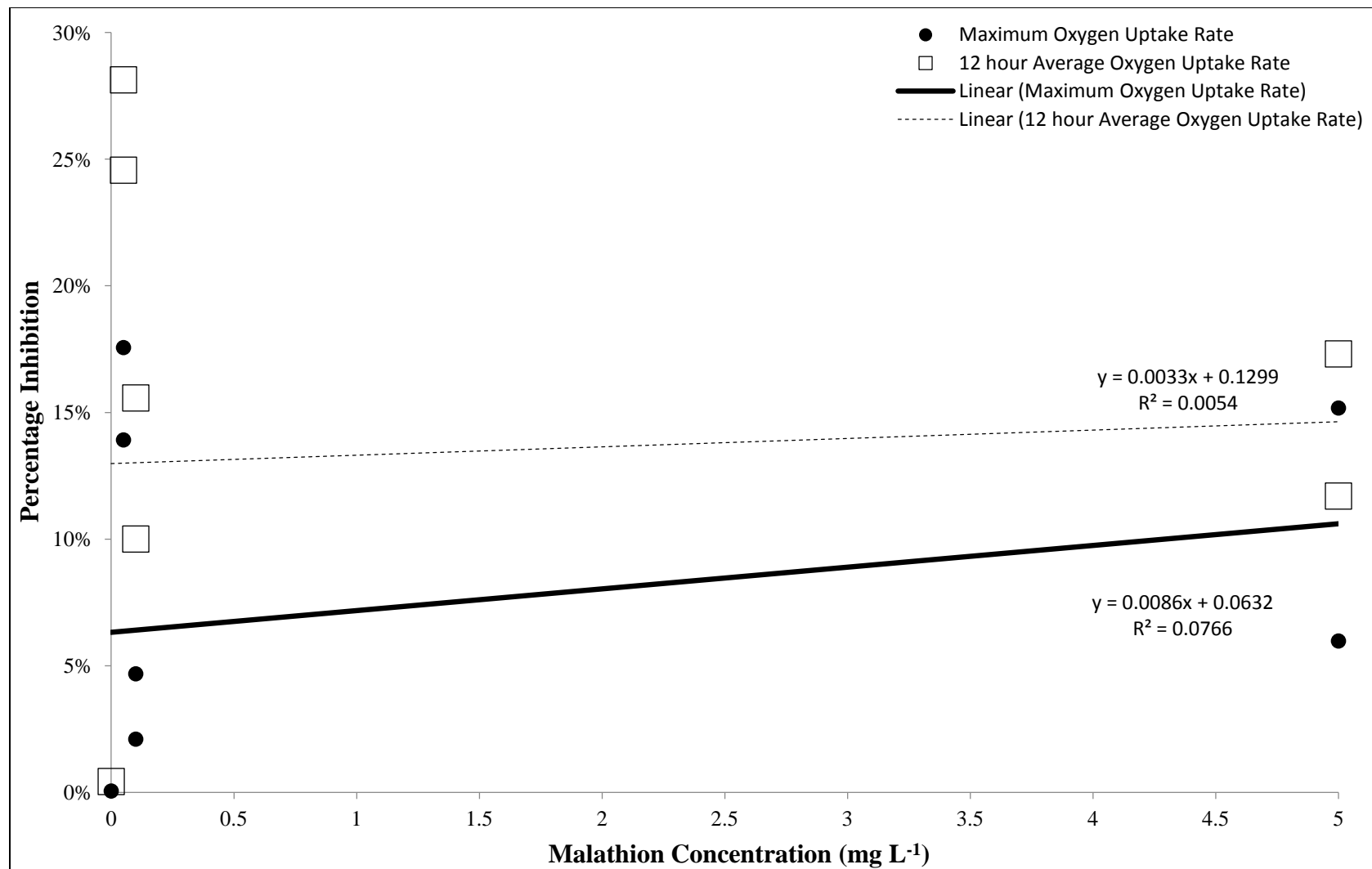


Figure 5: Respirometry Test 6 – Respiration Inhibition

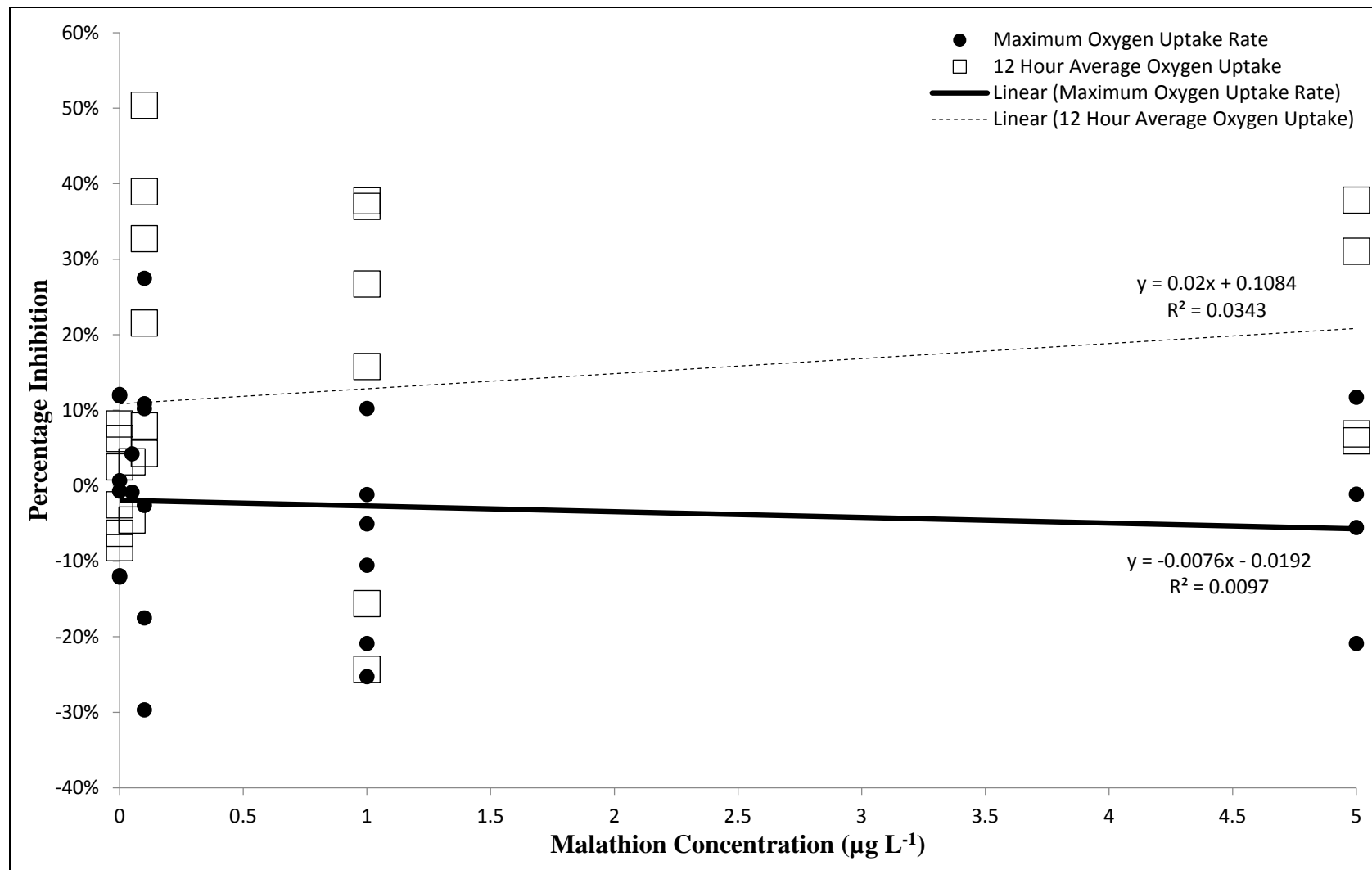


Figure 6: Respirometry Experiments (1 – 3) – Respiration Inhibition

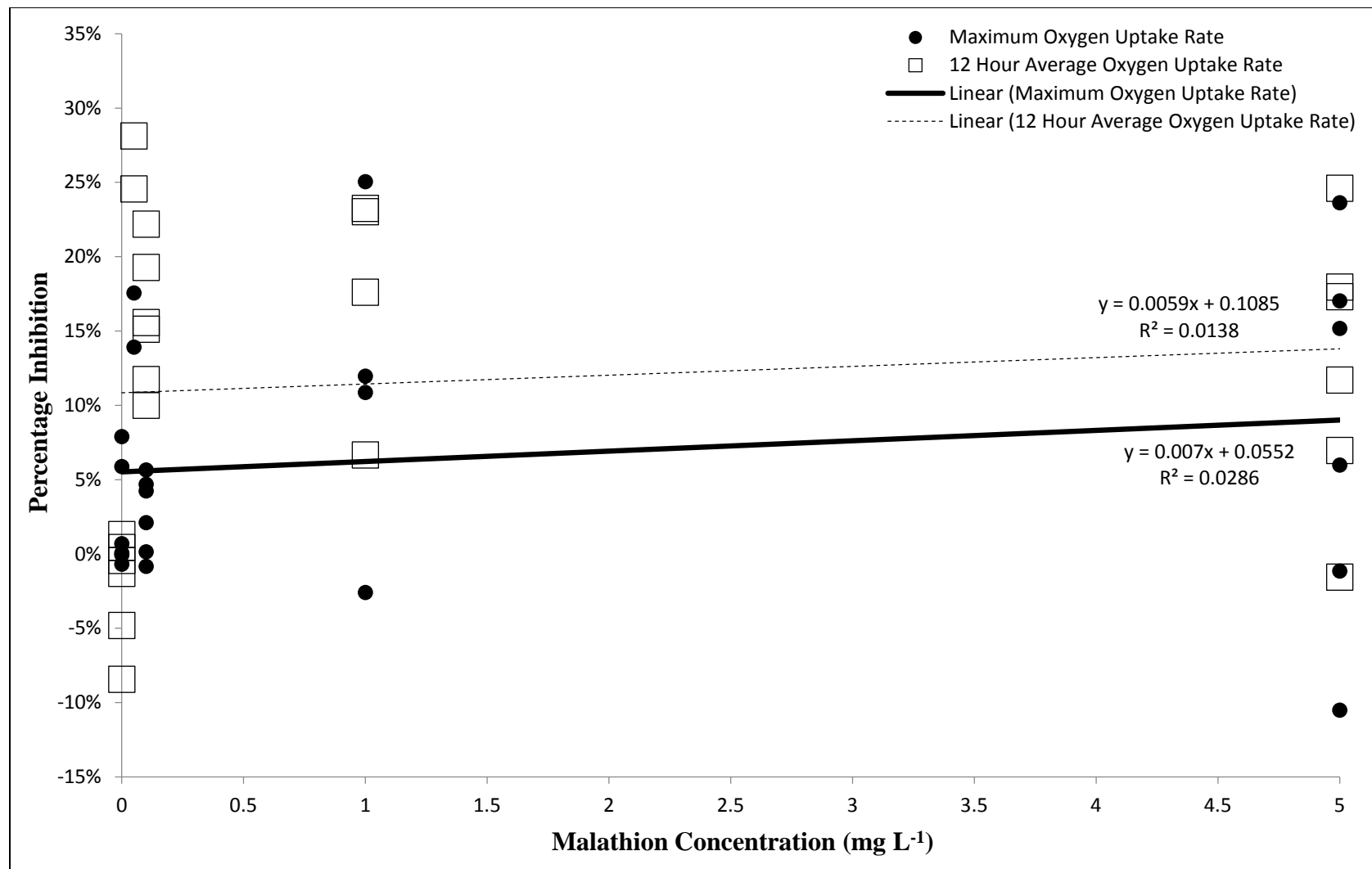


Figure 7: Respirometry Experiments (4 – 6) – Respiration Inhibition

The overall respirometry results indicate that the presence of malathion either has little effect or produces a decrease in the respiration rate of activated sludge with no discernable trends associated with the concentration of malathion. This variability in respiration inhibition between samples suggests that a direct relationship between the concentration of malathion and the respiration rate may not exist.

Utilizing the peak respiration rate for each sample taken during respirometry experiment 6, neither the substrate utilization model, Equation 6, nor the inhibition model, Equation 7, produced linearization lines with significant correlation to the data, Figure 8. The linearization of the utilization model and the inhibition model produced an  $R^2$  of 0.0766 and 0.066 respectively. The low  $R^2$  values for both models further supports the hypothesis that malathion is not acting singularly as a substrate that inhibits or promotes respiration.

$$r = r_o + kC \quad (6)$$

Where

$r = \text{respiration rate } (\mu\text{g min}^{-1})$

$r_o = \text{peak respiration rate in the absence of malathion } (\mu\text{g min}^{-1})$

$k = \text{respiration coefficient } (L \text{ min}^{-1})$

$C = \text{malathion concentration } (\mu\text{g L}^{-1})$

$$r = \frac{r_o}{\left(1 + \frac{C}{K_i}\right)} \quad (7)$$

Where

$r$  = respiration rate ( $\mu\text{g min}^{-1}$ )

$r_o$  = peak respiration rate in the absence of malathion ( $\mu\text{g min}^{-1}$ )

$C$  = malathion concentration ( $\text{mg L}^{-1}$ )

$K_i$  = inhibition coefficient ( $\text{mg L}^{-1}$ )

Examination of the other 5 respirometry experiments supports conclusions drawn from the data in experiment 6; see Table 8. Of the 6 experiments, 4 produced  $R^2$  values smaller than 0.1 with the highest  $R^2$  value being produced by experiment 5 with values of 0.4936 and 0.4686 for the utilization and inhibition models, respectively. The overall low predictability of both of these models when compared to the measured data suggests that malathion could be acting as both a source of microbial respiration while simultaneously inhibiting respiration for concentrations at or below  $5 \text{ mg L}^{-1}$ .

**Table 8: Substrate Utilization and Inhibition Linearization  $R^2$  Values**

Respirometry Test	Utilization Model $R^2$	Inhibition Model $R^2$
1	0.0363	0.0503
2	0.0255	0.0475
3	0.0198	0.0152
4	0.2888	0.2499
5	0.4936	0.4686
6	0.0766	0.0660

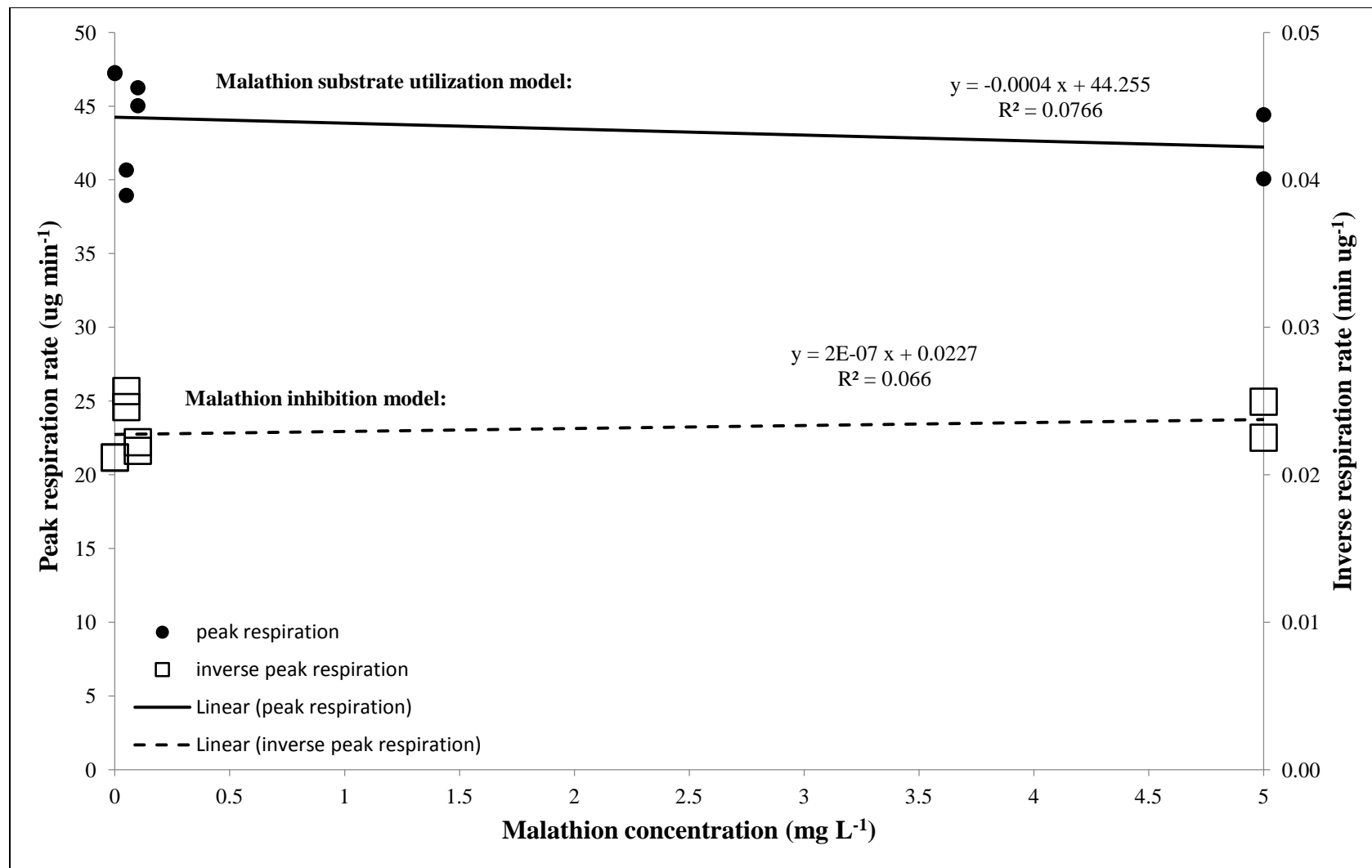


Figure 8: Respirometry Test 6 – Substrate Utilization & Inhibition Linearization Plots



The additive kinetics model is based upon the application of the dual substrate model listed in Equation 8. Typical representation of the model indicates microbial growth ( $\mu$ ). However, to facilitate model implementation within our given data, respiration which is associated with growth, was substituted into the model. To further simplify the model, it was assumed that the baseline respiration rate, which is dependent on substrate utilization without malathion present, would be consistent through all samples. This assumption allowed for the control samples average respiration rate to be substituted for the first set of terms in the additive kinetics model. The second set of terms was replaced with an Andrews Equation (Equation 9) which allows for the modeling of a substrate that inhibits its own biodegradation. The modified additive kinetics model is shown in Equation 10.

Within the model, use of a low  $K_s$  indicates that the substrate has a high affinity to encourage growth or respiration. The use of a low  $K_i$  indicates that the substrate has a high affinity to inhibit growth or respiration. Values were chosen for  $K_s$  and  $K_i$  that allowed the model to produce results that most closely resembled that measured data. While this approach does not allow for an extremely accurate application of the model, it does provide an indication as to the relative relationship between  $K_s$  and  $K_i$ . For example,  $K_i$  being lower than  $K_s$  is an indication that malathion causes greater inhibition of respiration than it does promoting respiration.

The results of model manipulation utilizing respiration rates from respirometry experiment 6 results in a  $K_i$  value of 1 and a  $K_s$  value of 100 to produce a result that is close to the measured data. Further raising  $K_s$  produces a lower predicted respiration rate

at a malathion concentration of  $5 \text{ mg L}^{-1}$ . However, very little change is noticed as  $K_s$  is increased as a value of 100 produces essentially a flat line.

The results indicated that in the low parts per million high values of  $K_s$  and low values of  $K_i$  produced results that were relatively close to the measured data. The results from the experiments in the low parts per billion required low values of both  $K_s$  and  $K_i$  for the model to produce results consistent with the measured data. At extremely low concentrations of malathion, this model will have limited use as the primary substrate driving respiration would greatly exceed the concentration of malathion and could potentially mask any effects the malathion might produce at such low concentrations.

The values of  $K_s$  and  $K_i$  required to closely represent the data for all 6 experiments can be seen in Table 9, graphical representation of the models can be found in Appendix B. The difficulty in consistently replicating the measured data with the application of the model utilizing similar values for  $K_i$  and  $K_s$  across all 6 experiments indicates that additional factors outside of the parameters identified in the model are potentially contributing to changes in respiration of the activated sludge during the 12 hours immediately following exposure.

**Table 9: Additive Kinetics Model Parameters**

Respirometry Test	Additive Kinetics model	
	$K_i$	$K_s$
1	1	1
2	1	.05
3	1	1
4	1	50
5	1	500
6	1	100

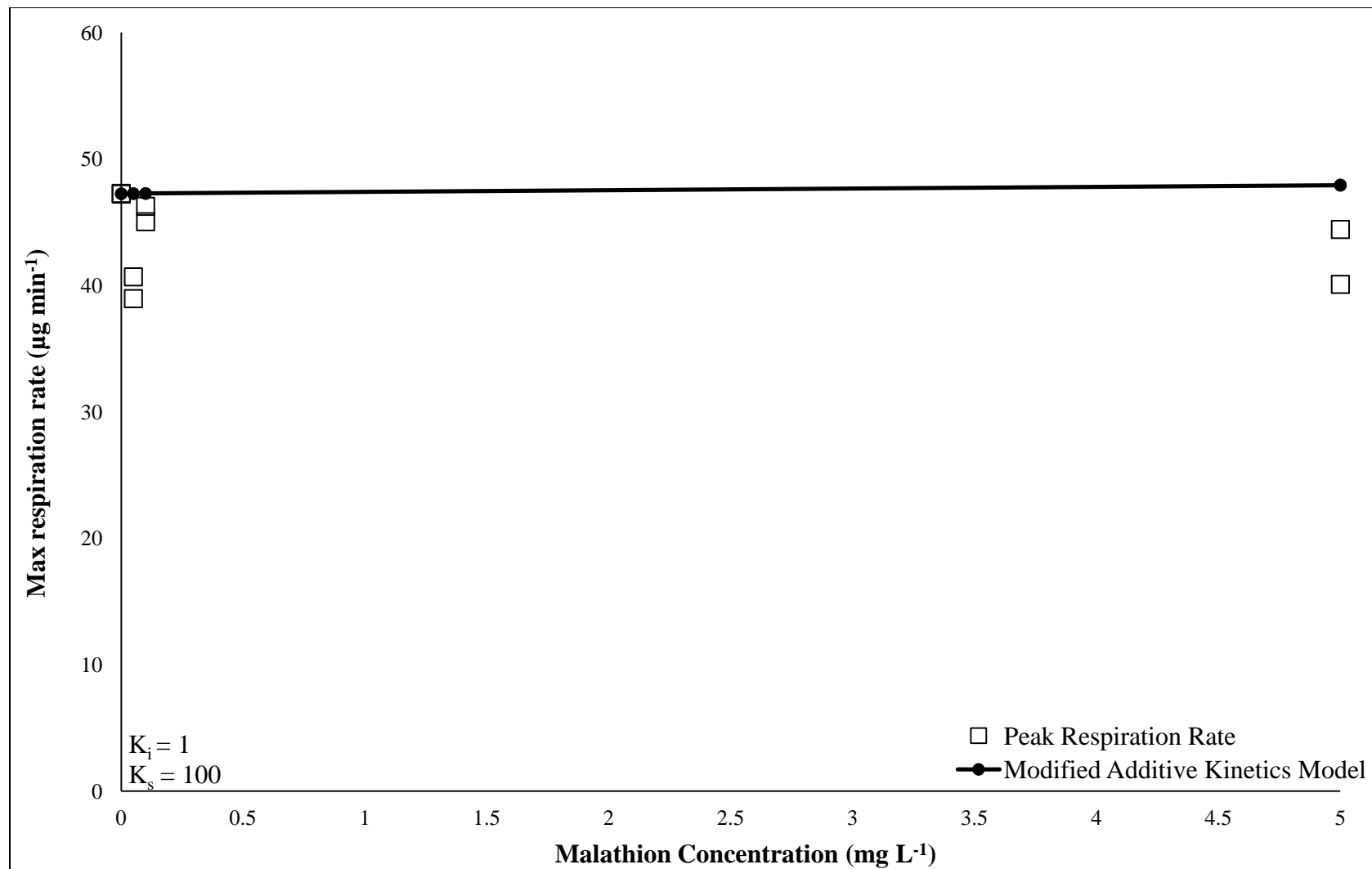


Figure 9: Respirometry Test 6 – Modified Additive Kinetics Model

$$\mu = \frac{\mu_{max} S_1}{K_1 + S_1} + \frac{\mu_{max_2} S_2}{K_2 + S_2} \quad (8)$$

(Blanch & Clark, 1997, p. 195)

Where

$\mu$  = specific growth rate ( $hrs^{-1}$ )

$\mu_{max}$  = maximum specific growth rate due to substrate 1 ( $hrs^{-1}$ )

$\mu_{max2}$  = maximum specific growth rate due to substrate 2 ( $hrs^{-1}$ )

$S_1$  = Concentration of substrate 1 ( $mg L^{-1}$ )

$S_2$  = Concentration of substrate 2 ( $mg L^{-1}$ )

$K_1$  = Substrate 1 half saturation or substrate-affinity constant ( $mg L^{-1}$ )

$K_2$  = Substrate 2 half saturation or substrate-affinity constant ( $mg L^{-1}$ )

$$\mu = \mu_{max} * \frac{S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \quad (9)$$

(TAZDAIT, et al., 2013)

Where

$\mu$  = specific growth rate ( $hrs^{-1}$ )

$\mu_{max}$  = maximum specific growth rate ( $hrs^{-1}$ )

$S$  = Substrate concentration ( $mg L^{-1}$ )

$K_s$  = Half saturation or substrate-affinity constant ( $mg L^{-1}$ )

$K_i$  = Substrate inhibition constant ( $mg L^{-1}$ )

$$R = R_o + R_{max} * \frac{S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \quad (10)$$

Where

*R = respiration rate*

*R<sub>o</sub> = Maximum respiration rate in the absence of malathion*

*R<sub>max</sub> = Maximum respiration rate in the presence of malathion*

*S = Concentration of malathion (mg L<sup>-1</sup>)*

*K<sub>s</sub> = Half saturation or substrate-affinity constant (mg L<sup>-1</sup>)*

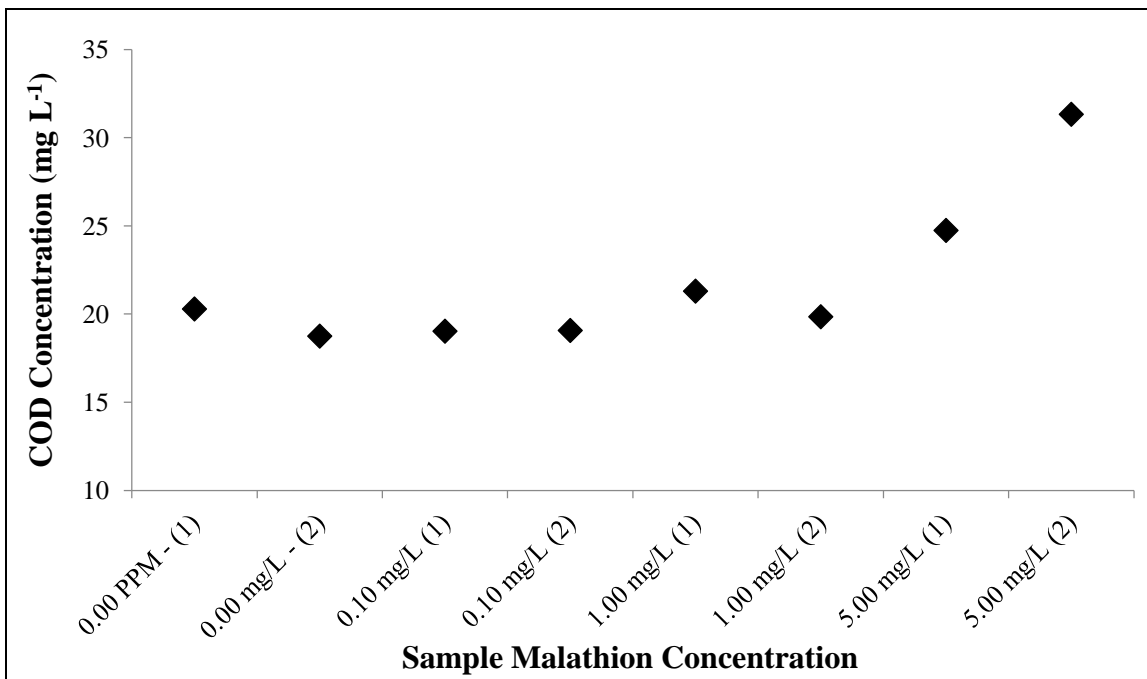
*K<sub>i</sub> = Substrate inhibition constant (mg L<sup>-1</sup>)*

The examination of the oxygen consumption data, the respiration inhibition, and substrate utilization models of activated sludge exposed to malathion did not produce any clear indication of a potential threshold concentration that would limit activated sludge's efficiency to continue to treat waste water treatment plant influent. The data did indicate a decrease respiration rate due to exposure to malathion, however, that is not necessarily an indication of poor performance and reduced effluent quality.

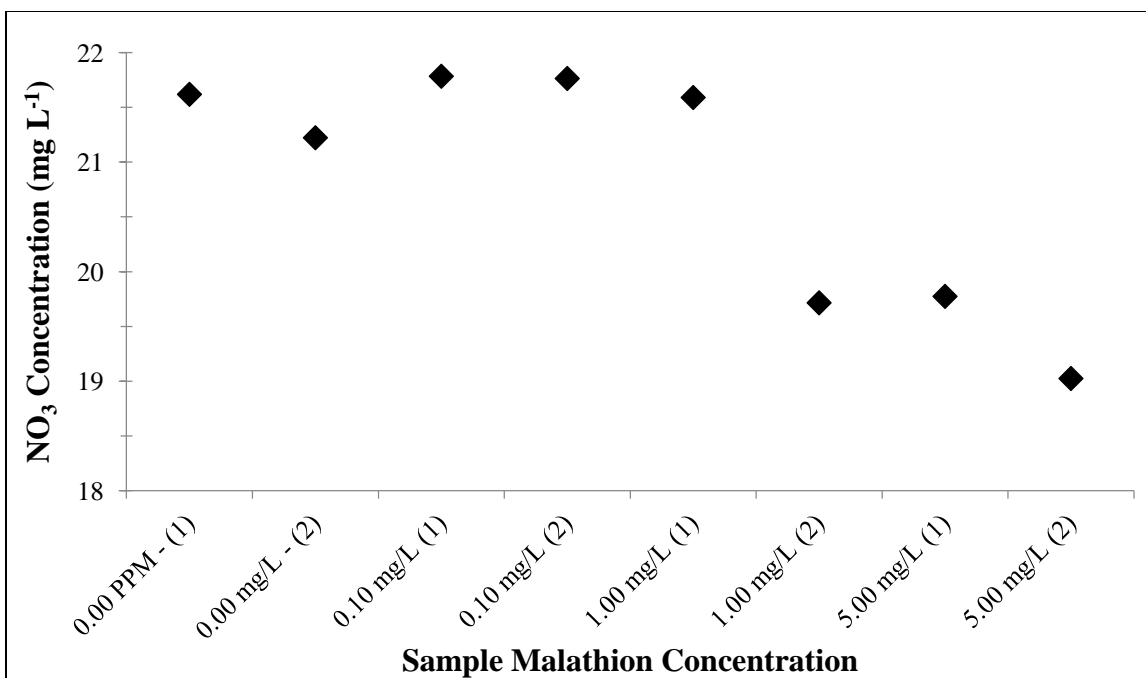
The lack of a clear threshold concentration for respiration data fails to provide a concentration to base the design of the short term and long term experiments on. However, examination of the final concentrations of COD and NO<sub>3</sub> for respirometry experiments 4 – 6 provided insight into a potential threshold concentration that could be used for additional experimentation. This data analysis is limited in that initial baseline data was taken from just two sample jars and was assumed to be consistent across all 8 samples within a given experiment. This assumption is generally supported by data

collected in the batch testing in which initial concentrations of all measured parameters, with one exception, were within 10% of the average initial starting concentration.

The results of COD concentration testing following respirometry experiment 4 indicated that malathion concentrations of  $5.00 \text{ mg L}^{-1}$  have the potential to limit the oxidation of COD during a twelve hour exposure as shown in Figure 10. Concentrations of malathion at or below  $1.00 \text{ mg L}^{-1}$  resulted in COD concentrations similar to the control with a value of  $19.6 \pm 1.03 \text{ mg L}^{-1}$  while a concentration of  $5 \text{ mg L}^{-1}$  of malathion resulted in a COD concentration of  $28.03 \pm 4.66 \text{ mg L}^{-1}$ . The increased COD concentrations were also observed in respirometry test 6.



**Figure 10: Respirometry Experiment 4 – Final COD Concentrations**



**Figure 11: Respirometry Experiment 4 – Final NO<sub>3</sub> Concentrations**

Similar to the results of COD oxidation, NO<sub>3</sub> production was negatively affected at malathion concentrations of 5.00 mg L<sup>-1</sup> as shown in Figure 11. Concentrations of malathion below 1.00 mg L<sup>-1</sup> resulted in NO<sub>3</sub> concentrations similar to the control with a value of  $21.59 \pm 0.68$  mg L<sup>-1</sup> while a concentration of 5 mg L<sup>-1</sup> of malathion resulted in a NO<sub>3</sub> concentration of  $19.40 \pm 0.66$  mg L<sup>-1</sup>. The decreased NO<sub>3</sub> concentrations were also observed in respirometry tests 5 and 6. The reduced COD oxidation and NO<sub>3</sub> production indicate that effluent quality could be negatively affected between 0.10 and 5.00 mg L<sup>-1</sup>. Janezcko, et al. (2014), indicated that negative effects to the effluent were observed at 5.00 mg L<sup>-1</sup>. Utilizing the data from Janezcko, et al. (2014) and the COD and NO<sub>3</sub> concentrations from the respirometry experiments led to the decision to use concentrations of 0.1 and 3.00 mg L<sup>-1</sup> to potentially bracket an inhibition threshold.

### ***Short-Term Degradation and Malathion Removal***

Short term malathion degradation results, depicted in Figure 12, showed that the malathion concentration decreased by a measured average of 93% when exposed to an initial designed concentrations of  $3.00 \text{ mg L}^{-1}$ , with a final concentration of  $361 \text{ } \mu\text{g L}^{-1}$  for batch test 3. Malathion concentration decreased below detectible limits by hour 11, based upon a minimum GC/MS signal to noise ratio of 10, when exposed to initial designed concentration of  $0.1 \text{ mg L}^{-1}$ . The shape of the malathion reduction curves show similarities when examined on appropriate scales. Each curve begins with a rapid removal of malathion within the first 2 hours, accounting for at least 50% of the total malathion removed. Following the initial degradation, a gradual decline in malathion concentrations existed between hours 2 and 12. The malathion degradation rate for reactor 3 during the initial 2 hours was  $0.55 \text{ mg L}^{-1} \text{ hr}^{-1}$ , the degradation rate for reactor 2 during the same time period was  $0.037 \text{ mg L}^{-1} \text{ hr}^{-1}$ . The degradation rates following the first 2 hours for reactors 2 and 3 were  $65.4$  and  $1.9 \text{ } \mu\text{g L}^{-1} \text{ hr}^{-1}$  respectively. Full results for batch test 3 can be found in Appendix F.

Malathion degradation results in batch test 1 and 2 showed similar trends with significant reduction of malathion during the initial 2 hours, followed by a relatively slow removal rate after hour 2. All three testes resulted in quantities below detection limits for reactor 2 prior to the completion of the 12 hour cycle. Similarly, all 3 tests resulted in reduction of malathion concentrations for reactor 3 to levels below  $400 \text{ } \mu\text{g L}^{-1}$  which is below the World Health Organizations recommended limit of  $900 \text{ } \mu\text{g L}^{-1}$  in drinking water (World Health Organization, 2004) but above the EPA drinking water guideline of  $200 \text{ } \mu\text{g L}^{-1}$  (ATSDR, 2003, p. 226).



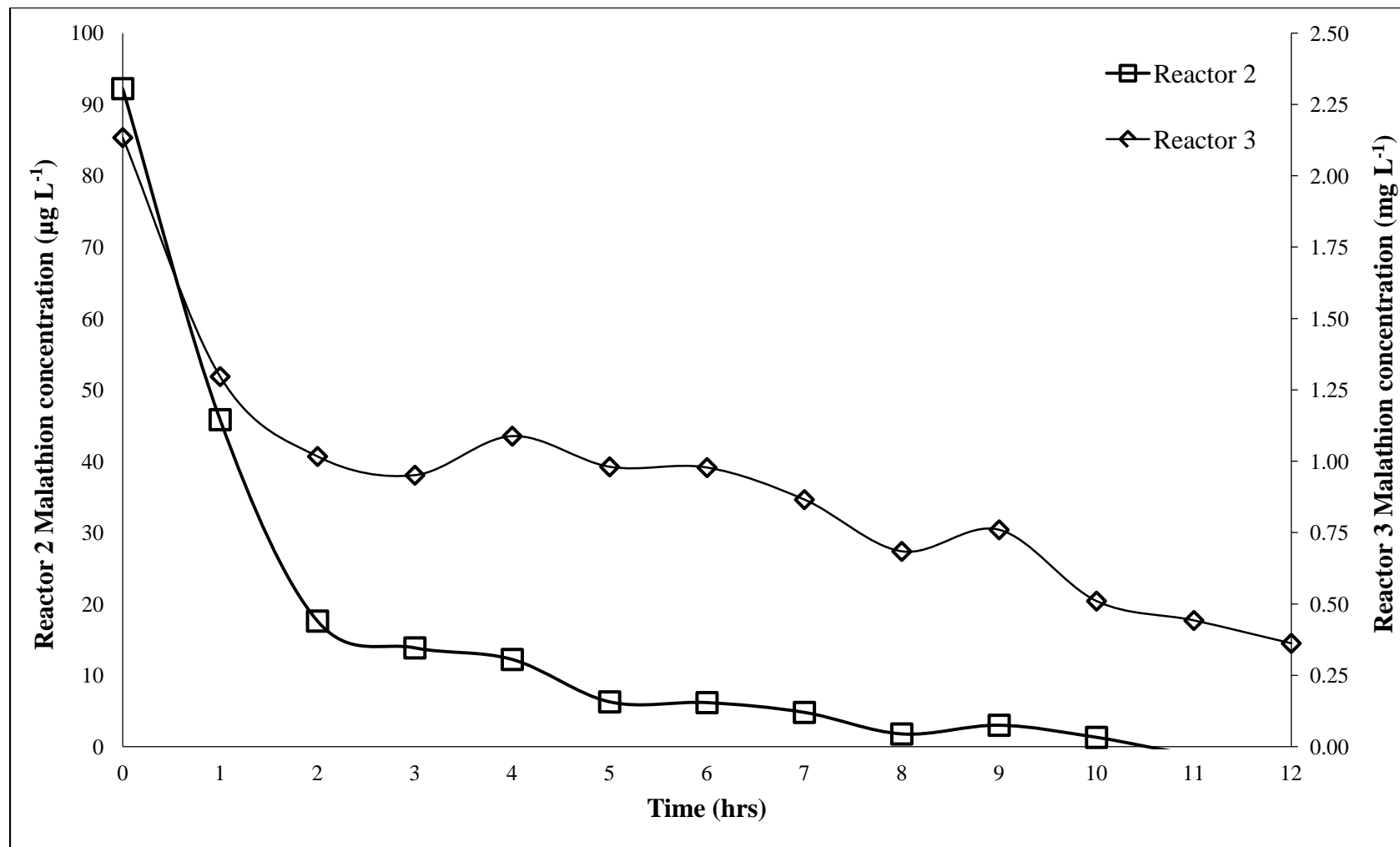


Figure 12: Batch Test 3 – Malathion Removal

The COD oxidation results for batch test 3 showed a decrease from a starting concentration of  $105 \pm 4.32 \text{ mg L}^{-1}$  to a final concentration of  $23.67 \pm 3.82 \text{ mg L}^{-1}$  after 12 hours. The COD oxidation rate when not exposed to malathion was  $34.47 \text{ mg L}^{-1} \text{ hr}^{-1}$ , when exposed to malathion the oxidation rate was approximately  $29 \text{ mg L}^{-1} \text{ hr}^{-1}$  during the first 2 hours. The shape of the COD oxidation curves were identical for all cases, beginning with a rapid oxidation of COD during the first 2 hours, followed by a gradual decline in oxidation rates between hours 2 and 4. Following hour 4, the COD oxidation rate when not exposed to malathion, was  $0.22 \text{ mg L}^{-1} \text{ hr}^{-1}$ ; when exposed to malathion, an average rate of  $0.75 \text{ mg L}^{-1} \text{ hr}^{-1}$  was observed.

All three batch tests indicated similar trends in COD reduction and final concentration; see Appendix C. One exception to this trend existed. The  $3.00 \text{ mg L}^{-1}$  reactor during test 2 had a final COD concentration of  $53.63 \text{ mg L}^{-1}$ . While the final concentration was significantly higher within this one sample, the overall percentage of COD removed was similar to all other samples. Reactor 3 in batch test 2 had an unusually high starting concentration of COD,  $123 \text{ mg L}^{-1}$ , when compared to the other two reactors which averaged  $80 \text{ mg L}^{-1}$ . This variation in COD concentrations could potentially be from incomplete mixing of feed B into the sample or COD remaining from partially completed oxidation reactions taking place in the sequencing batch reactors which provided the batch test sample sludge.

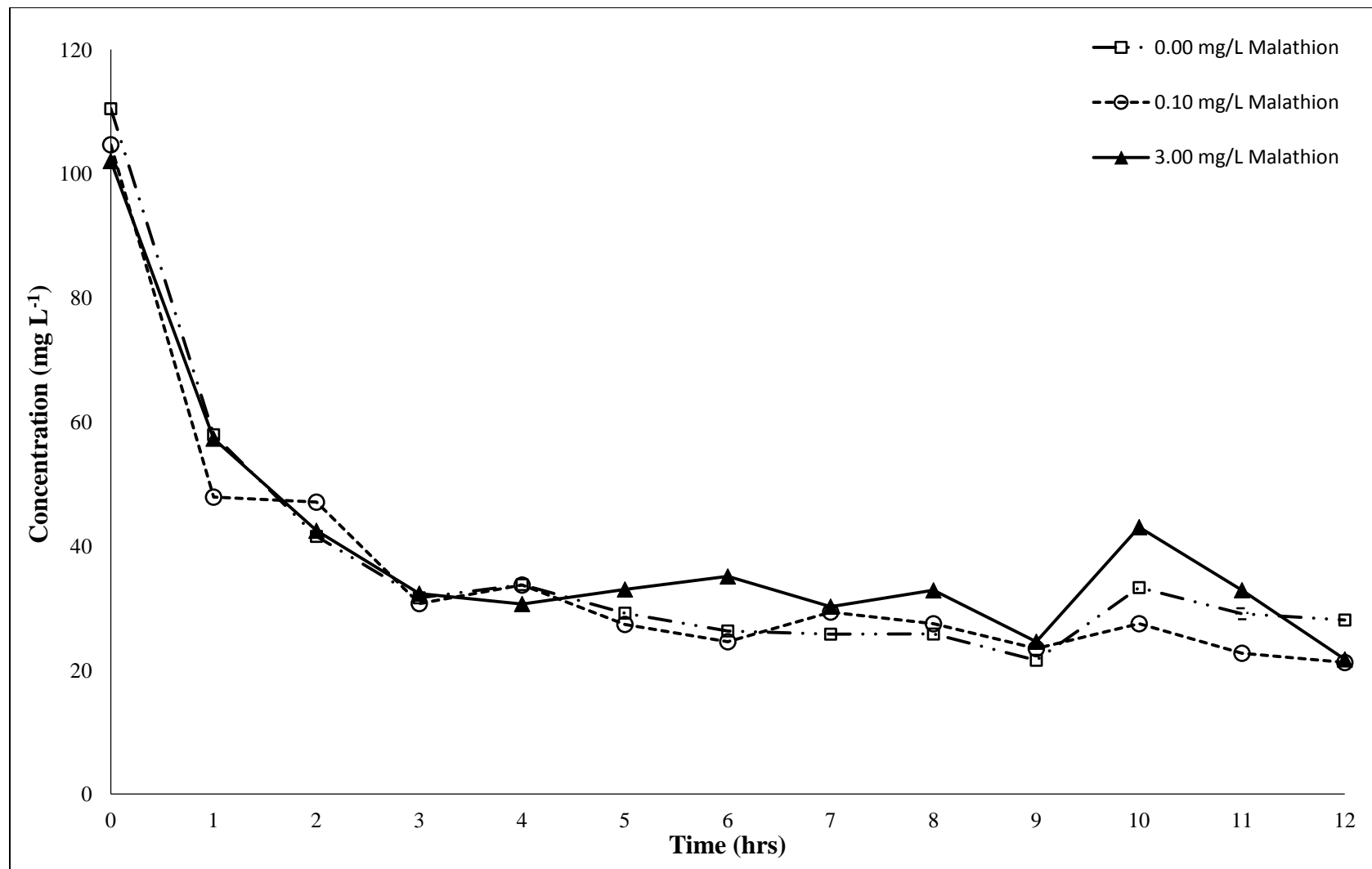


Figure 13: Batch Test 3 – COD Removal

Similar to COD, batch test 3  $\text{NH}_3$  concentrations decreased from starting values of  $14.3 \pm 0.24 \text{ mg L}^{-1}$  to a concentration of  $4.318 \pm 0.208 \text{ mg L}^{-1}$  by the end of the 12 hour cycle in the third batch test.  $\text{NH}_3$  reduction curves showed similar trends across all three malathion concentrations with nearly identical starting and finishing values. The curves remained relatively stable during the initial 2 hours. Nitrification began following hour 2 with reactors 1, 2, and 3 having distinctly different removal rates until approximately hour 10. The concentrations between hour 10 and 12 remained relatively constant for all three reactors with a nearly identical final concentration, Figure 14.

The peak  $\text{NH}_3$  reduction rate was approximately  $1.4 \text{ mg L}^{-1} \text{ hr}^{-1}$  starting at hour 2 and ending at hour 9 when the sludge was not exposed to malathion. When the sludge was exposed to  $0.10 \text{ mg L}^{-1}$  of malathion, the  $\text{NH}_3$  reduction rate was approximately  $2.0 \text{ mg L}^{-1} \text{ hr}^{-1}$  between hours 2 and 7. The  $\text{NH}_3$  reduction rate when exposed to  $3.00 \text{ mg L}^{-1}$  of malathion peaked between hours 2 and 10 with average rate of  $1.24 \text{ mg L}^{-1} \text{ hr}^{-1}$ .

The inability to completely remove all  $\text{NH}_3$  during the third test could potentially be attributed to the lower quantity of TSS and VSS during the experiment. While the final concentrations of all three experiments did not indicate that malathion had an effect on the overall systems performance when compared to the control, the rate of  $\text{NH}_3$  reduction did vary between the different concentrations of malathion, with a greater variation indicated as the concentration of TSS was reduced. Batch test 2 produced similar results as batch test 3, indicating that activated sludge exposed to  $3.00 \text{ mg L}^{-1}$  malathion had a slower utilization rate of  $\text{NH}_3$  than the reactor with  $0.10 \text{ mg L}^{-1}$  malathion, which had utilization rates similar to the control sample, see Appendix C.

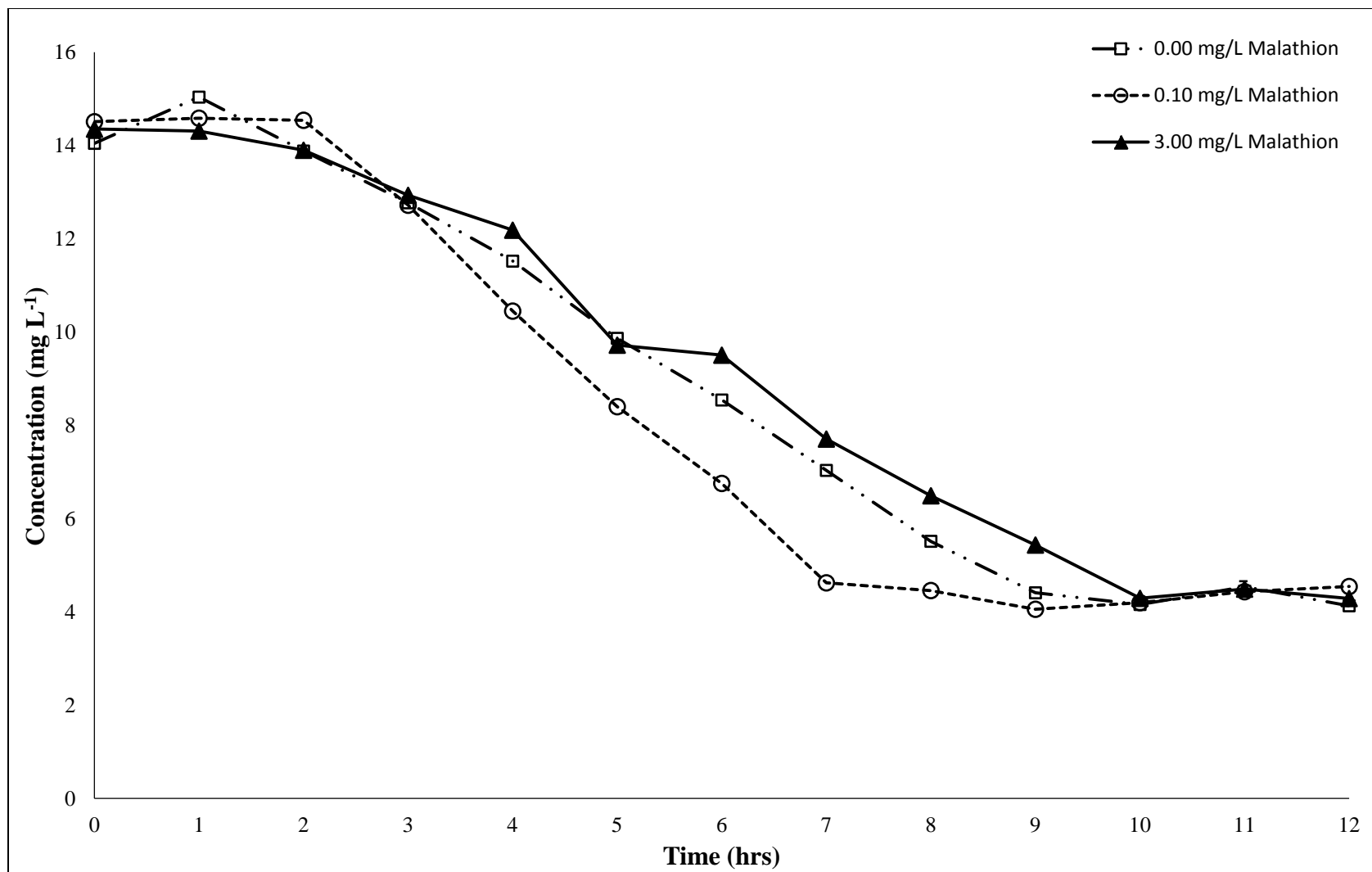


Figure 14: Batch Test 3 – NH<sub>3</sub> Removal

The results of batch test 3 showed that  $\text{NO}_3$  production proceeded at a rate of  $0.90 \text{ mg L}^{-1} \text{ hr}^{-1}$  in the absence of malathion. When malathion was added at a concentration of  $0.1 \text{ mg L}^{-1}$  and  $3.0 \text{ mg L}^{-1}$ , the production of  $\text{NO}_3$  proceeded at a rate of  $0.98$  and  $0.87 \text{ mg L}^{-1} \text{ hr}^{-1}$ , respectively. The shape of the  $\text{NO}_3$  production curve maintained a relatively constant slope and similar shape throughout the 12 hour experiment. Across all three concentrations of malathion, an initial average concentration of  $12.61 \pm 0.21 \text{ mg L}^{-1} \text{ NO}_3$  was recorded with a final concentration of  $23.15 \pm 0.39 \text{ mg L}^{-1} \text{ NO}_3\text{-N}$  (Figure 15). Batch tests 1 and 2 produced similar results with all three concentrations of malathion producing  $\text{NO}_3$  at vary similar rates during the experiment with peak concentrations and changes in the rate of production of  $\text{NO}_3$  all being achieved at similar times during a given experiment.

This data indicates overall that during the initial 12 hours of exposure to these concentrations, malathion had no noticeable inhibition on the overall nitrification or COD oxidation process. There was a noticed difference in the rate of  $\text{NH}_3$  removal, potentially associated with TSS concentrations, as a result of exposure to malathion. However, all three reactors reached the same final  $\text{NH}_3$  concentrations within the 12 hour reaction cycle. Finally, while variations existed in the final COD concentrations, activated sludge's ability to perform COD oxidation appeared to be unaffected, based upon the percentage of COD removal over the 12 hour period.

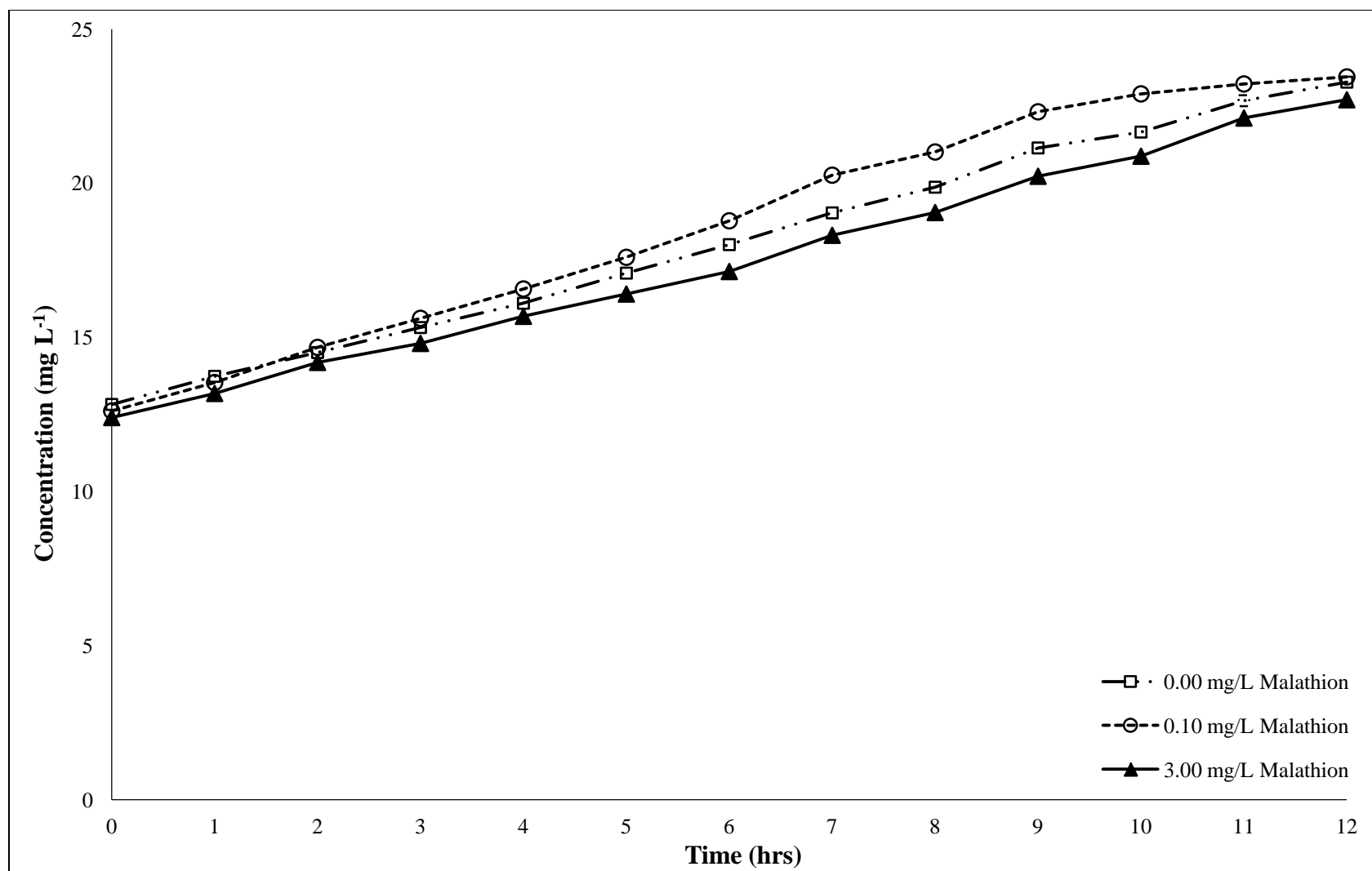


Figure 15: Batch Test 3 – NO<sub>3</sub> Production

### ***Long-Term Degradation and Malathion Removal Monitoring***

During the long term malathion exposure experiment, the concentration of malathion in the effluent generated by each reactor was below the detectable limit of  $1 \mu\text{g L}^{-1}$  for the GC/MS. This was unexpected as Janezcko, et al., (2014) indicated that at the initial reactor concentrations of  $5 \text{ mg L}^{-1}$ , effluent concentrations of malathion peaked at 4.5 days with an increase in the effluent concentration correlated with longer retention times. It is important to note that malathion concentration samples were not taken for three days following addition of malathion to reactor 3. It is possible, based upon the batch test results, that detectible quantities of malathion were present prior to the first sample taken 84 hours after initial exposure.

Figure 16, sample file 81, shows the GC/MS chromatograph results for a  $1 \text{ mg L}^{-1}$  malathion in methanol check sample which had a retention time of 15.499 minutes and area under the peak of 48,672 and a signal to noise ratio of 9214.8. Figure 17, sample file 36, shows a typical GC/MS chromatograph results indicative of all effluent samples taken over the 30 day period for both reactors exposed to malathion. Sample 36 was the first recover from reactor 3, on 7 Dec 14, 84 hours following the addition of malathion. The GC/MS sample retention time was 15.494 minutes with an average area of 23, 2 samples were tested, and an average signal to noise ratio of 3.05. The 7 Dec 14 samples' low signal to noise ratio and extremely small indicate that if malathion is present within the sample it is below the detection limit of  $1 \mu\text{g L}^{-1}$ .



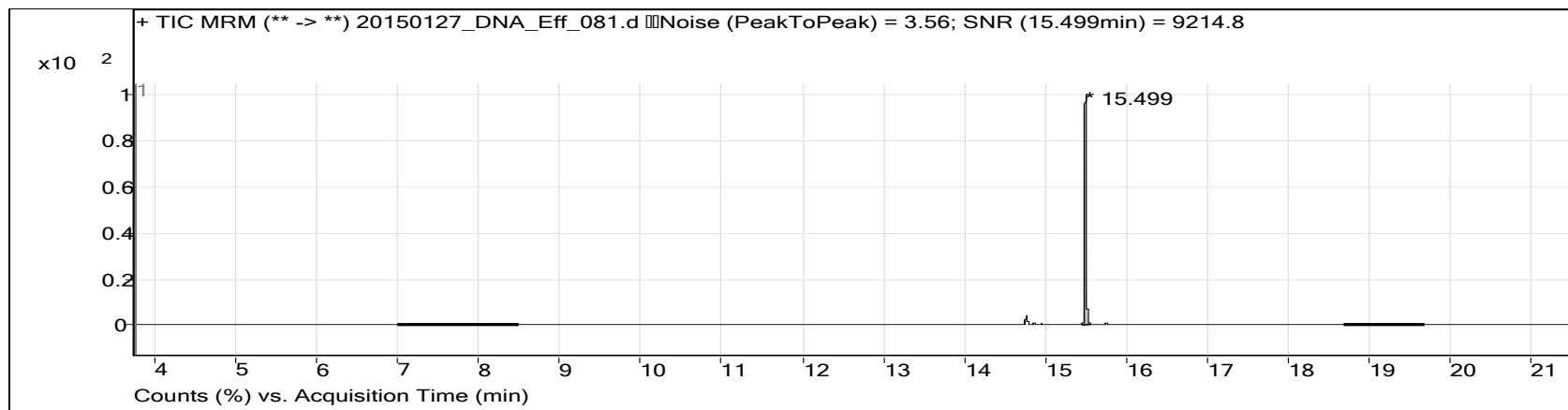


Figure 16: GC/MS Results for 1.00 mg L<sup>-1</sup> Check Sample (Sequence #81)

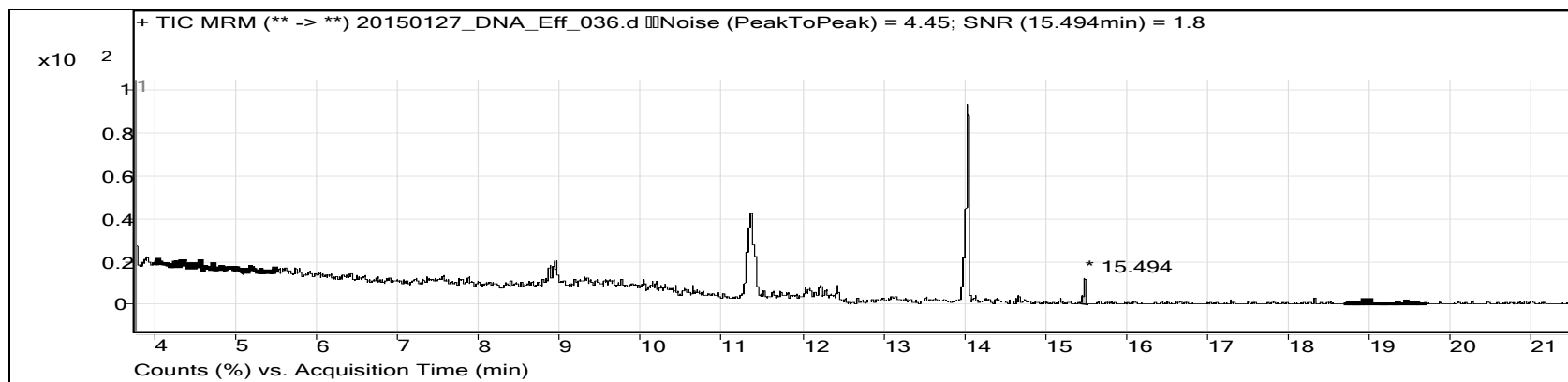
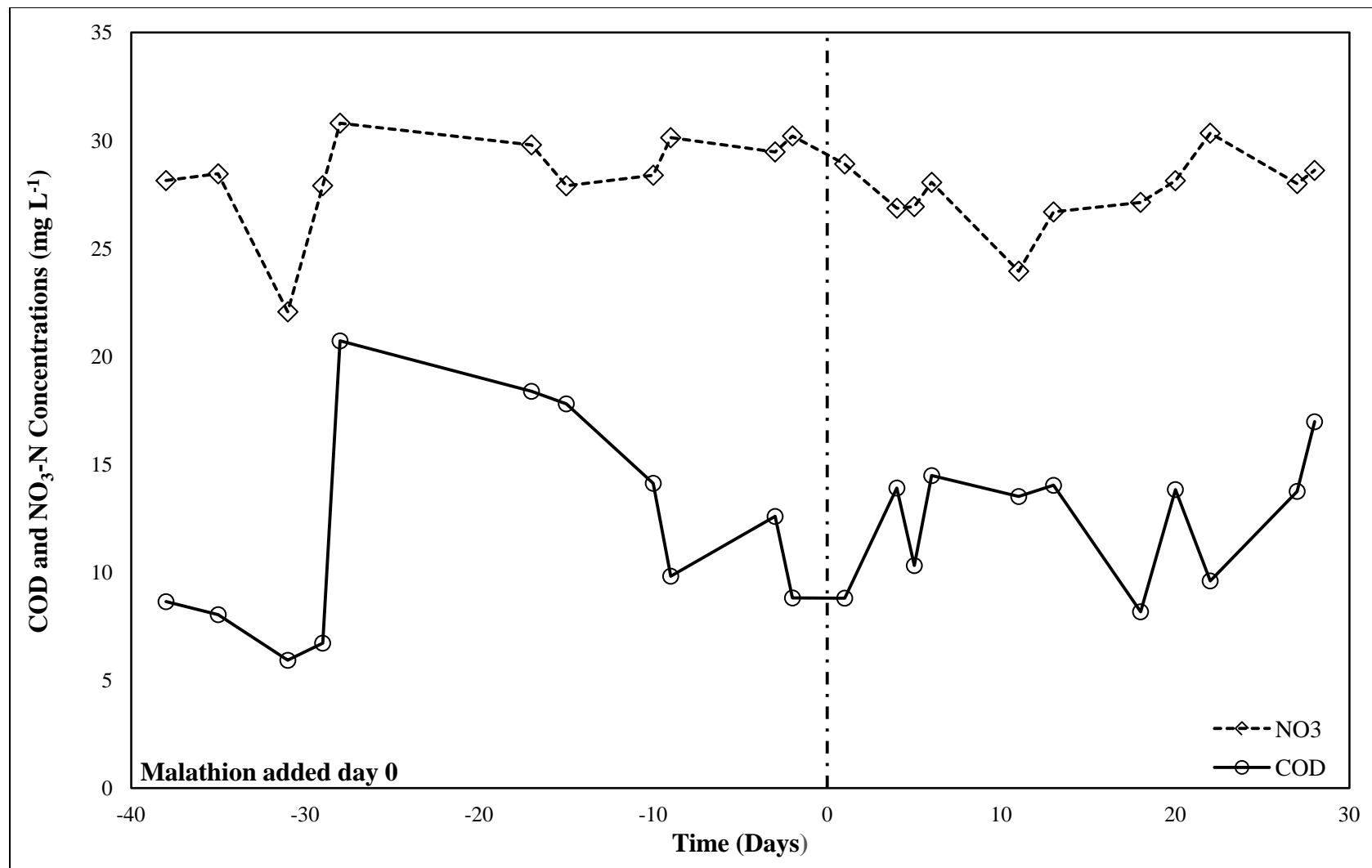


Figure 17: GC/MS Results for Reactor 3, Day 2 (7 Dec 14 - Sequence #36)

Examining the COD oxidation in the long-term reactors provided insight into the effects of malathion on activated sludge efficiency. The activated sludge in reactor 1, with an initial malathion concentration of  $0.10 \text{ mg L}^{-1}$ , was unaffected by the presence of malathion. Prior to the addition of malathion, the mean effluent COD concentration was  $11.95 \pm 26.16 \text{ mg L}^{-1}$ . After the addition of malathion, the mean effluent COD concentrations were  $12.47 \pm 7.82 \text{ mg L}^{-1}$ . A two tailed t-test, assuming unequal variance, indicated that there was no significant ( $\alpha = 0.1$ ) difference in effluent COD concentrations ( $p = 0.7686$ ) when comparing the 11 samples taken before and the 11 samples taken after the addition of malathion. Utilizing the same statistical test,  $\text{NO}_3$  production also did not have any significant changes ( $p = 0.3239$ ) in the mean  $\text{NO}_3$  concentrations preceding and following the addition of malathion. Mean effluent concentrations of  $\text{NO}_3$  prior to the addition of malathion were  $28.47 \pm 5.77 \text{ mg L}^{-1}$ , and mean effluent concentrations following exposure to malathion were  $27.59 \pm 2.63 \text{ mg L}^{-1}$  (Figure 18).

The lack of a statistically significant change in both the COD oxidation and the  $\text{NO}_3$  production indicate that at a concentration of  $0.10 \text{ mg L}^{-1}$ , the heterotrophic bacteria and nitrifying bacteria were unaffected by the exposure to malathion. Variations within the rate of oxidation and or nitrification may exist, as indicated by the batch test results. However, the overall process results, which are indicative of the values that would be observed at a treatment plants discharge point and the most important indication of performance, were unaffected.



**Figure 18: Effluent COD and NO<sub>3</sub> Concentrations Pre and Post Malathion Exposure – Reactor 1 (0.10 mg L<sup>-1</sup>)**

The results from the activated sludge exposed to  $3.00 \text{ mg L}^{-1}$  shows that the mean effluent COD concentrations prior to malathion exposure of was  $12.09 \pm 21.36 \text{ mg L}^{-1}$ , and following exposure, the effluent COD concentrations were  $20.54 \pm 148.56 \text{ mg L}^{-1}$ . The large standard deviation can be attributed to the significant spike in effluent COD concentration that was observed on day 6. The observed spike at day 6 is consistent with the results from Janeczko, et al. (2014) that saw a spike in effluent concentration between days 3 and 8 when activated sludge was exposed to  $5 \text{ mg L}^{-1}$ .

Utilizing the same t-test as reactor 1, assuming unequal variance, there was a statistically significant difference ( $\alpha = 0.1$ ) detected in the mean effluent concentration of COD ( $p = 0.0291$ ) prior to and following the exposure to malathion. Additional testing, utilizing the same t-test parameters, indicated that if the COD spike on 11 Dec was removed, there was still a statistically significant difference in the mean COD concentrations prior to and following exposure to malathion. This exposure also statistically effected  $\text{NO}_3$  production ( $p = 0.0831$ ). The mean effluent concentrations prior to malathion exposure was  $29.03 \pm 1.35 \text{ mg L}^{-1}$  and following malathion exposure the mean effluent concentrations of  $\text{NO}_3\text{-N}$  were  $26.46 \pm 24.97 \text{ mg L}^{-1}$ , Figure 19. A statistically significant change in both the COD and the  $\text{NO}_3$  concentrations in the effluent indicate that at a concentration of  $3.00 \text{ mg L}^{-1}$ , the both the heterotrophic bacteria and nitrifying bacteria were negatively affected by the exposure to malathion.

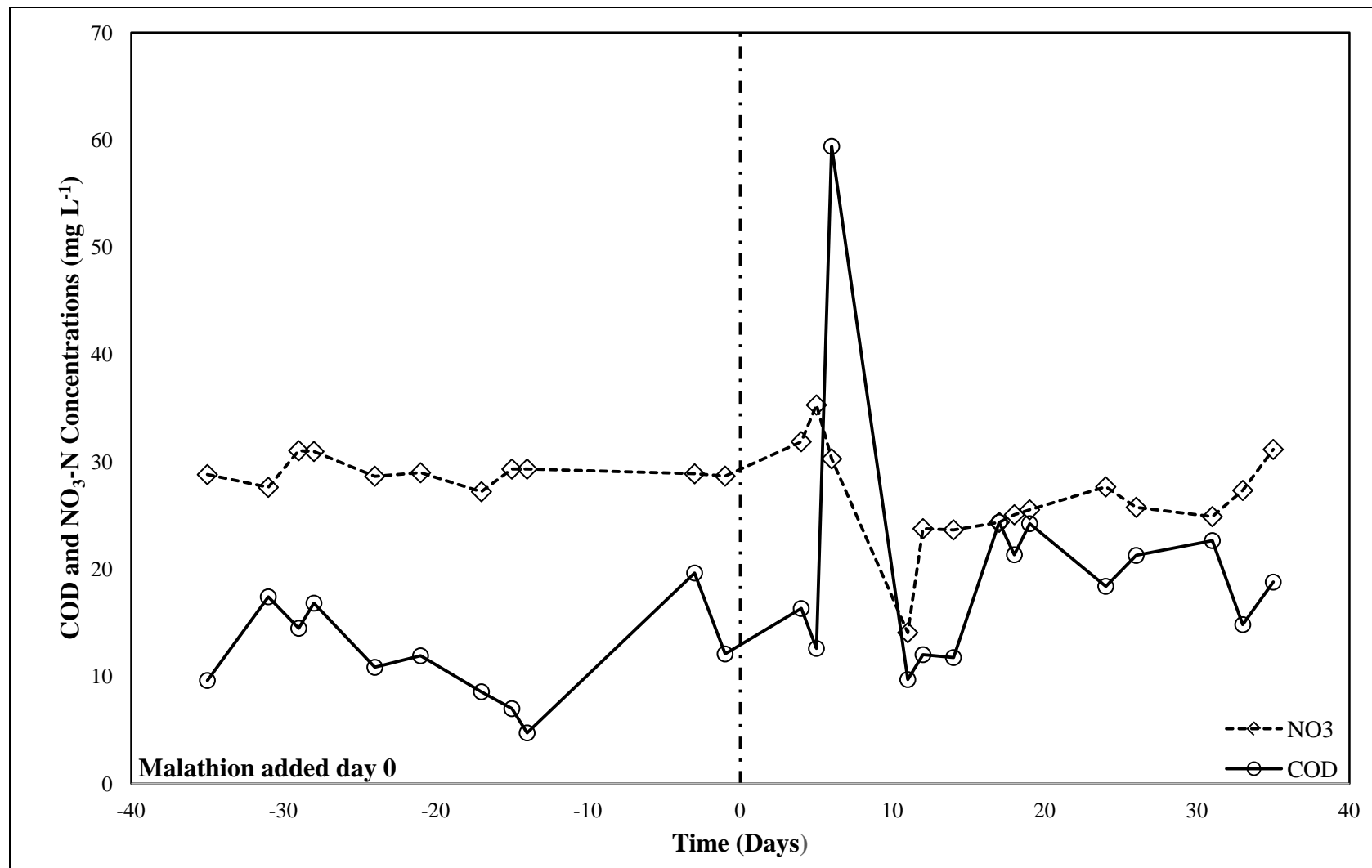


Figure 19: Effluent COD and NO<sub>3</sub> Concentrations Pre and Post Malathion Exposure – Reactor 3 (3.00 mg L<sup>-1</sup>).

## Conclusions

This study provides insight into the effect of malathion, a VX surrogate, on the activity and performance of activated sludge in a municipal wastewater treatment plant and demonstrates that concentrations of 3.00 and 0.1 mg L<sup>-1</sup> can be tolerated by an activated sludge wastewater treatment facility with no significant change to the effluent quality during the initial twelve hours of exposure. However, long-term exposure to 3.0 mg L<sup>-1</sup> does have a statistically significant effect on effluent quality. Additionally, it was demonstrated through respirometry testing that malathion concentrations between 1 – 5 mg L<sup>-1</sup> which can have a long term effect on effluent quality produce no discernable respiration trends of activated sludge, other than a general suppression of respiration due to malathion exposure. Furthermore, it was shown that malathion was degraded by at least 83% and 99+% for reactor concentration levels of 3.00 mg L<sup>-1</sup> and 0.10 mg L<sup>-1</sup> during the initial 12 hours of exposure and that over a continual long term exposure to the same concentrations, no detectable levels of malathion were observed in the effluent.

As a result, this study concludes that wastewater treatment facilities can continue to operate without any degradation to activated sludge performance at concentrations as high as 3.00 mg L<sup>-1</sup> for up to 12 hours and can operate for up to 30 days at concentrations of 0.10 mg L<sup>-1</sup> with no significant effect on effluent water quality. Furthermore, at an operating concentration of 3.00 mg L<sup>-1</sup>, detectable quantities of malathion could be expected in the plant effluent, dependent on sludge age and hydraulic retention times, immediately following initial exposure. After a short adaptation period, effluent concentrations of malathion should be well below detectable limits.

Limited research is available with respect to the respiration rate of activated sludge when exposed to malathion. However, Aragon et al., (2010) demonstrated that it is possible that low levels of exposure to a manganese(II) can produce an increase in respiration, where high levels inhibit respiration. It is possible that a similar situation exists with malathion.

Additionally the ability to effectively degrade malathion utilizing activated sludge is consistent with the research of both Janeczko, et al., (2014) and Ghoshdastidar, et al., (2012) with one noticeable exception. In previous research, an acclimation period has been observed, which has involved an increase in the concentration of malathion found in the effluent when exposed to influent concentrations of 3 mg L<sup>-1</sup> and higher. The acclimation period lasted between 2 and 10 days before achieving malathion reduction efficiency's greater than 90%. The results from this experiment did not indicate the presence of an acclimation period. It is possible that not sampling the effluent during the initial two days of exposures resulted in missing the acclimation period. However, both Janeczko, et al. (2014) and Ghoshdastidar, et al. (2012) indicate that acclimation periods were at least 5 to 10 days. The lack of an acclimation period could be the result of identifying a concentration that does not required an adjustment period. It is also possible that the lack of acclimation could stem from slightly different operating conditions and influent parameters creating a bacterial community that was much more tolerant and able to adapt much more rapidly to the introduction of malathion.

In addition to the malathion degradation, short term experiments yielded similar results to Janeczko, et al., (2014) with respect to activated sludge's ability to continue to degrade NH<sub>3</sub> when exposed to malathion. However, there was a significant difference in

the reduction of COD and the production of  $\text{NO}_3$  during the similar 12 hour batch test experiments. The results indicated an initial rapid decrease in COD with continual oxidation occurring though the full 12 hours. Janeczko, et al., (2014) indicated the opposite with an increase in COD due to the exposure to malathion. Additionally, the production of  $\text{NO}_3$  reported by Janeczko, et al., (2014) was significantly higher despite similar  $\text{NH}_3$  reduction reactions. As with the long term removal of malathion, variations in the microbial community could explain differences in their ability to adapt to the exposure of malathion. It is also possible that different bacterial strains are able to effectively utilize malathion as a substrate at various concentrations.



### **III. Conclusion**

#### **Chapter Overview**

This chapter discusses the findings identified while addressing the research questions that were posed in Chapter 1. The review of findings section provides a summation of the in depth discussion, located in the scholarly article followed by brief discussion of the significance and limitations of the research. Finally, areas of future research are discussed followed by a summary of this thesis.

#### **Review of Findings**

The research results show that the effects of malathion on activated sludge's ability to continue to process influent wastewater between  $0.1 \text{ mg L}^{-1}$  and  $3.00 \text{ mg L}^{-1}$  is limited. Both concentrations demonstrated a capability to reduce the oxygen consumption rate of activated sludge. Even while operating with less oxygen consumption; it was shown that there was no detectible difference in nitrification or COD oxidation efficiency during the initial 12 hours of exposure. Over a 30-day exposure period, however, at a concentration of  $3.00 \text{ mg L}^{-1}$ , both nitrification and COD oxidation showed statistically significant ( $\alpha = 0.1$ ) inhibition. In contrast to an exposure to a concentration of  $0.1 \text{ mg L}^{-1}$ , which continued to produce effluent at quality similar to what was produce pre malathion exposure. Finally, this work also demonstrated that during continual exposure to malathion, at the above concentrations, may result in the degradation of the primary toxicant to below detectible levels prior to the effluent being discharged.

## **Significance of Research**

In the response to a chemical warfare incident, decontamination efforts will need to be timely to ensure minimal exposure. A widely accepted decontamination procedure is to use copious amounts of low-pressure water. Water from the decontamination efforts could potentially be sent to a wastewater treatment facility for processing. This research demonstrates that concentrations in the high parts per billion range can be safely processed within an activated sludge treatment system without significant effects to the effluent water quality from both the perspective of standard wastewater effluent quality metrics looking at COD, NH<sub>3</sub>, NO<sub>2</sub>, and NO<sub>3</sub> concentrations but also organophosphate contamination as well.

## **Limitations**

The use of malathion as a surrogate for research into the effects of VX on an activated sludge based sewage treatment system allows for the close approximation of many of the physical and chemical characteristics of VX. However, while similar, the differences between malathion and VX only provide an approximation of the interactions that may be expected. Additionally, lab conditions are designed to simplify field conditions to facilitate a controlled and reproducible experiment. Therefore, the lab scale results should only be used to create a general understanding of the possible interactions of organophosphates within an operational system. Parameters that were well controlled within the laboratory such as influent characteristics, temperature, and toxic load are subject to change within a municipal treatment plant. Significant changes in any one of

these parameters could have an impact on the bacterial community resulting in a bacterial population that would be unable to reproduce the results seen in the lab.

Both VX and malathion have hydrolysis pathways that are pH and temperature dependent, with compounds of various toxicity resulting as a result of the hydrolysis pathway utilized. The presence of toxic hydrolysis products such as malaoxon or iso-malathion, may produce inhibition similar to or more severe than malathion and knowledge of their concentrations within the effluent could provide insight into experimental results. However, no abiotic malathion degradation products were identified as this was outside the scope of the original research question.

### **Future Research**

The research suggests that malathion may be used as a substrate with minimal to no impact to overall performance at low concentrations. The manipulation of the synthetic wastewater to systematically eliminate phosphorus and sulfur sources from the influent would provide additional insight into the level at which activated sludge is able to metabolize malathion when it is the only source of required trace elements.

A second area of future research is the examination of the long-term recovery profile based upon a variety of exposure concentrations and exposure times within the activated sludge. While direct impacts to effluent quality are important, an experiment examining the short term impact and long term recovery due to short duration high concentration exposures would provide insight into expected recovery times and long term viability of a wastewater treatment system exposed to chemical warfare agents. A high concentration short term exposure would replicate the bulk of all decontamination

waste water reaching the treatment plant at one time and being processed as quickly as possible. Similar quantities of contamination diluted over an extended period of time would replicate purposeful isolation of contamination as it enters the waste water treatment facility, followed by its slow reintroduction into the process stream at concentrations believed to be low enough to not impact short term effluent quality or long term plant operations.

Understanding long term effects is important, as this research has shown concentrations of malathion that fail to impact short term quality still have the potential to induce enough of a sustained stress response to negatively affect treatment plant operations over a much longer scale.

## **Summary**

This research examined the impact and fate of malathion, a VX surrogate, in a municipal wastewater treatment plant utilizing activated sludge. The intent of this research was to identify the threshold concentration of malathion that would limit the activated sludge's ability to continue to produce effluent within the ranges prescribed in the plants pollution discharge permit. The research methodology involved respirometry, twelve hour bench scale batch testing, and long term exposure testing using activated sludge grown and maintained in three sequencing batch reactors seeded from the Fairborn Water Reclamation Facility.

Data showed that at concentrations below  $3.00 \text{ mg L}^{-1}$ , respiration rates were negatively affected by malathion concentrations when compared to the control. This would limit the applicability of inline respiration testing for toxicant detection.

Additionally, it was determined that during the initial 12 hours of exposure, effluent quality measured by COD,  $\text{NH}_3$ , and  $\text{NO}_3$  concentrations indicated no negative impact. Furthermore, it was shown that long term exposure to concentrations of  $3.00 \text{ mg L}^{-1}$  of malathion resulted in a decrease in both COD removal and  $\text{NO}_3$  production. Finally, it was shown that during initial exposure, the reduction of malathion concentrations reached  $96.62 \pm 6.714\%$ , with long term exposure effluent concentrations of malathion never exceeding the detection the limit of  $5 \text{ } \mu\text{g L}^{-1}$ .

Overall, this research indicates that an activated sludge based wastewater treatment systems may be able to effectively degrade organophosphate based chemical warfare agents similar to malathion for periods up to 12 hours with concentrations as high as  $3.00 \text{ mg L}^{-1}$ , and for as long as 30 days with concentrations as high as  $0.10 \text{ mg L}^{-1}$  with no impact to effluent quality.

## Appendix A: Feed Chemicals

Chemical Name	Chemical Formula	Manufacture	Lot No.	MW (g mol <sup>-1</sup> )
Sodium Bicarbonate	NaHCO <sub>3</sub>	Fisher Scientific		84.01
Peptone		Remel	031606	
Sodium Acetate Trihydrate	C <sub>2</sub> H <sub>3</sub> NaO <sub>2</sub> • 3H <sub>2</sub> O	Fisher Scientific	990232	136.08
Ammonium Chloride	NH <sub>4</sub> Cl	Acros Organics	A0290146	53.49
Magnesium Chloride	MgCl <sub>2</sub> • 6H <sub>2</sub> O	Fisher Scientific	990351	203.31
Calcium Chloride	CaCl <sub>2</sub> • 2H <sub>2</sub> O	Fisher Scientific	986659	147.02
Potassium dihydrogen phosphate	KH <sub>2</sub> PO <sub>4</sub>	Research Chemicals Ltd	87807A	136.09
Citric Acid	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	Acros Organics	A0297228	192.13
Hippuric Acid	C <sub>9</sub> H <sub>9</sub> NO <sub>3</sub>	Acros Organics	A0257679	179.17
NTA trisodium salt	C <sub>6</sub> H <sub>6</sub> NNa <sub>3</sub> O <sub>6</sub> • H <sub>2</sub> O	Acros Organics	A0272764	275.09
EDTA tetrasodium salt	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>8</sub> Na <sub>4</sub> • 2H <sub>2</sub> O	Fisher Scientific	107263	416.2
Boric Acid	H <sub>3</sub> BO <sub>3</sub>	Fisher Scientific	102615	61.83
Potassium Iodide	KI	Acros Organics	A0266044	166.00
Ferric Chloride Hexahydrate	FeCl <sub>3</sub> • 6H <sub>2</sub> O	Fisher Scientific	103005	270.3
Zinc Sulfate Heptahydrate	ZnSO <sub>4</sub> • 7H <sub>2</sub> O	Fisher Scientific	106404	287.56
Manganese Chloride Tetrahydrate	MnCl <sub>2</sub> • 4H <sub>2</sub> O	Fisher Scientific	101852	197.91
Copper(II) Sulfate Pentahydrate	CuSO <sub>4</sub> • 5H <sub>2</sub> O	Acros Organics	A0289795	249.68
Sodium molybdate (VI) dihydrate	Na <sub>2</sub> MoO <sub>4</sub> • 2H <sub>2</sub> O	Acros Organics	A0289594	241.95
Cobalt(II) chloride hexahydrate	CoCl <sub>2</sub> • 6H <sub>2</sub> O	Acros Organics	B0130899	237.93
Nickelous Chloride Hexahydrate	NiCl <sub>2</sub> • 6H <sub>2</sub> O	Fisher Scientific	AD-10362-24	237.71
Sodium tungstate dehydrate	Na <sub>2</sub> WO <sub>4</sub> • 2H <sub>2</sub> O	Acros Organics	A0284287	329.85

## Appendix B: Respirometry Data

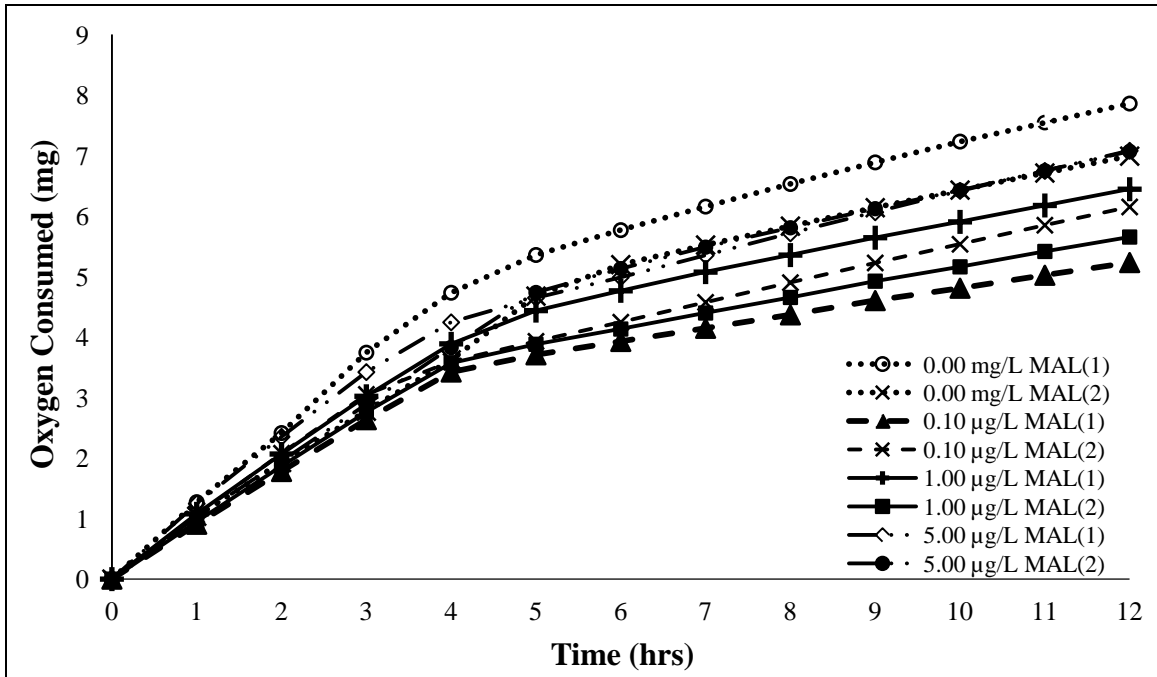


Figure 20: Respirometry Test 1 – Cumulative Oxygen Consumption

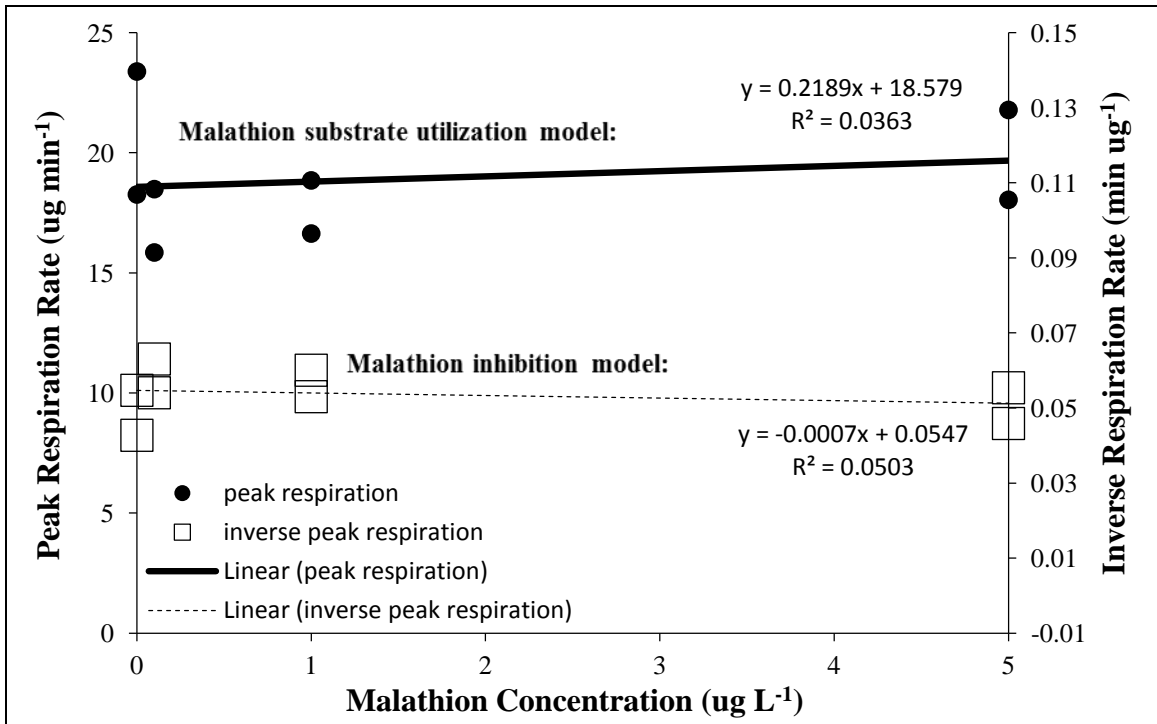


Figure 21: Respirometry Test 1 – Substrate Utilization & Inhibition Linearization Plots

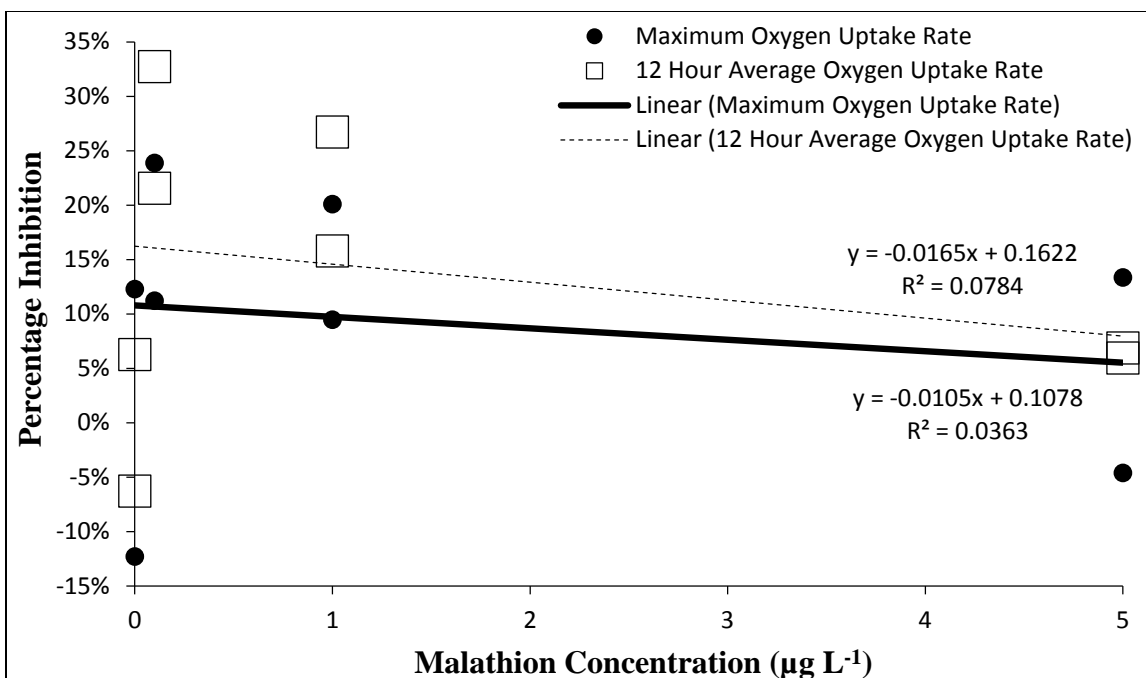


Figure 22: Respirometry Test 1 – Percentage Inhibition

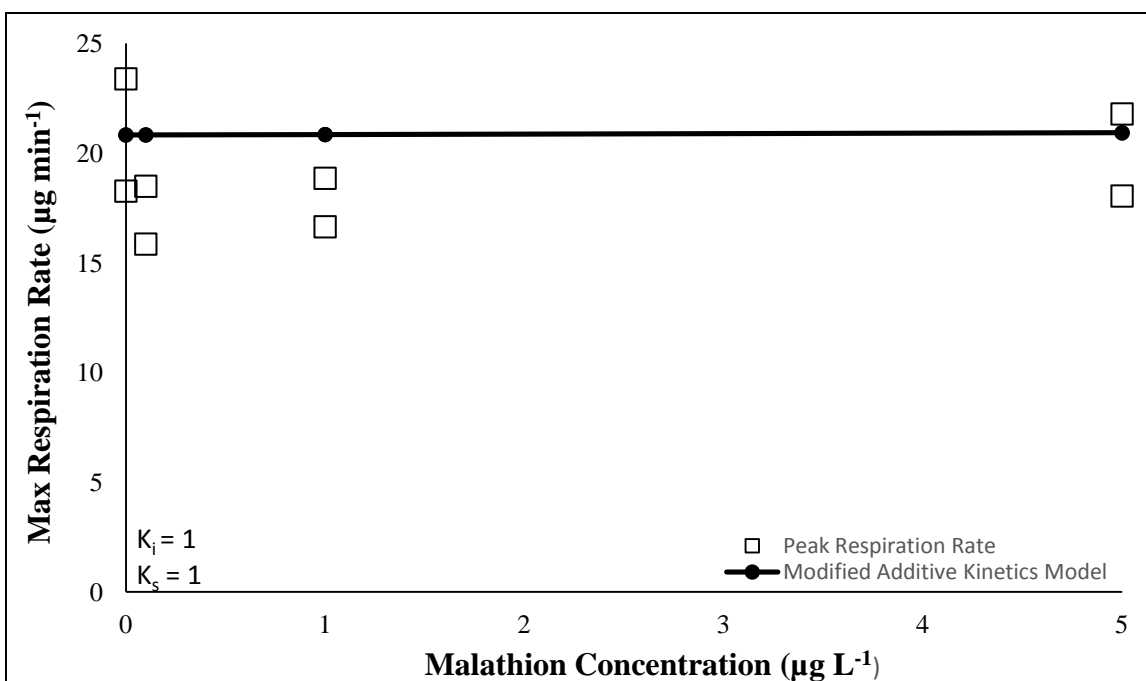


Figure 23: Respirometry Test 1 – Additive Kinetics Model



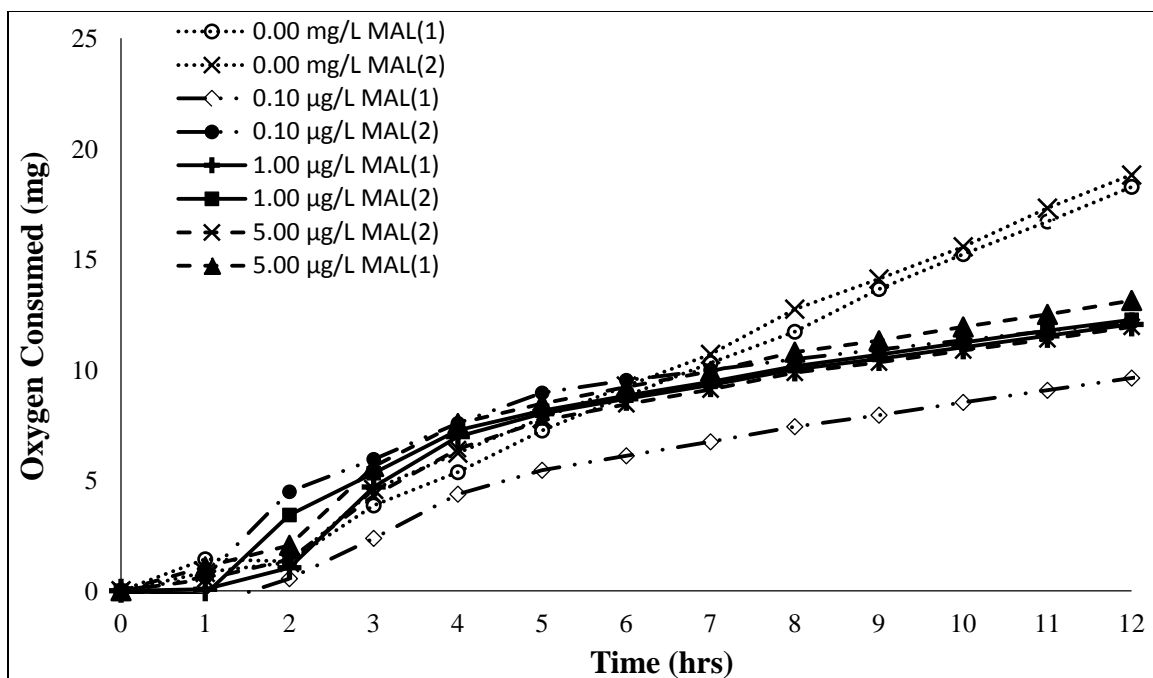


Figure 24: Respirometry Test 2 – Cumulative Oxygen Consumption

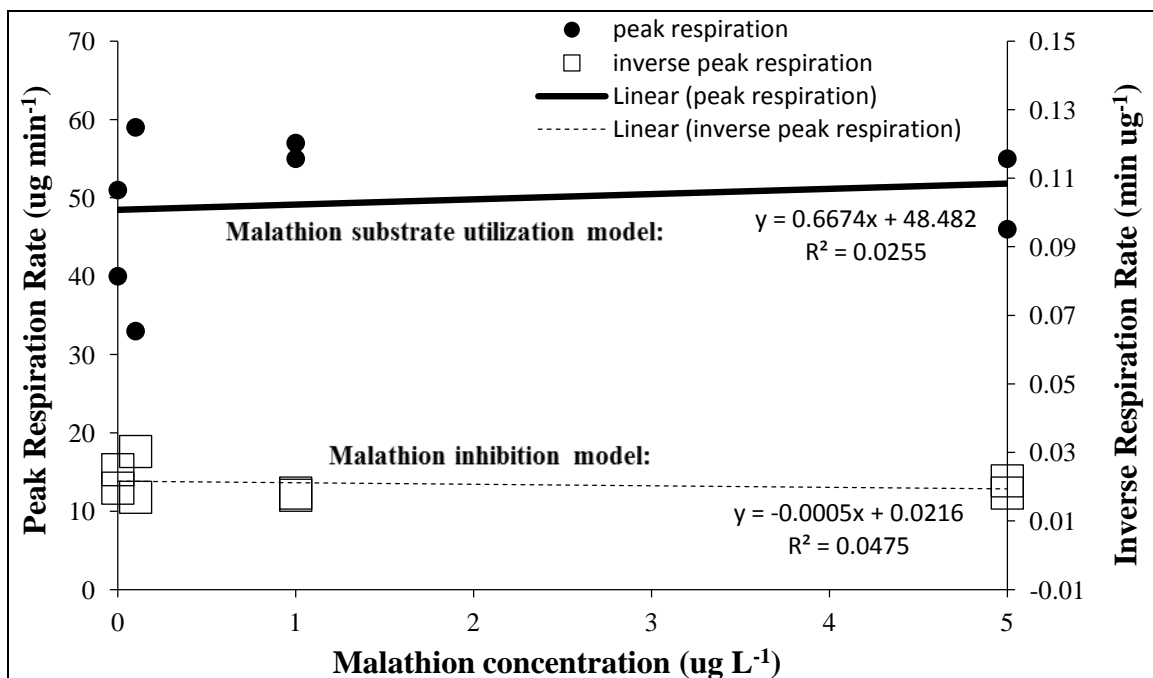


Figure 25: Respirometry Test 2 – Substrate Utilization & Inhibition Linearization Plots

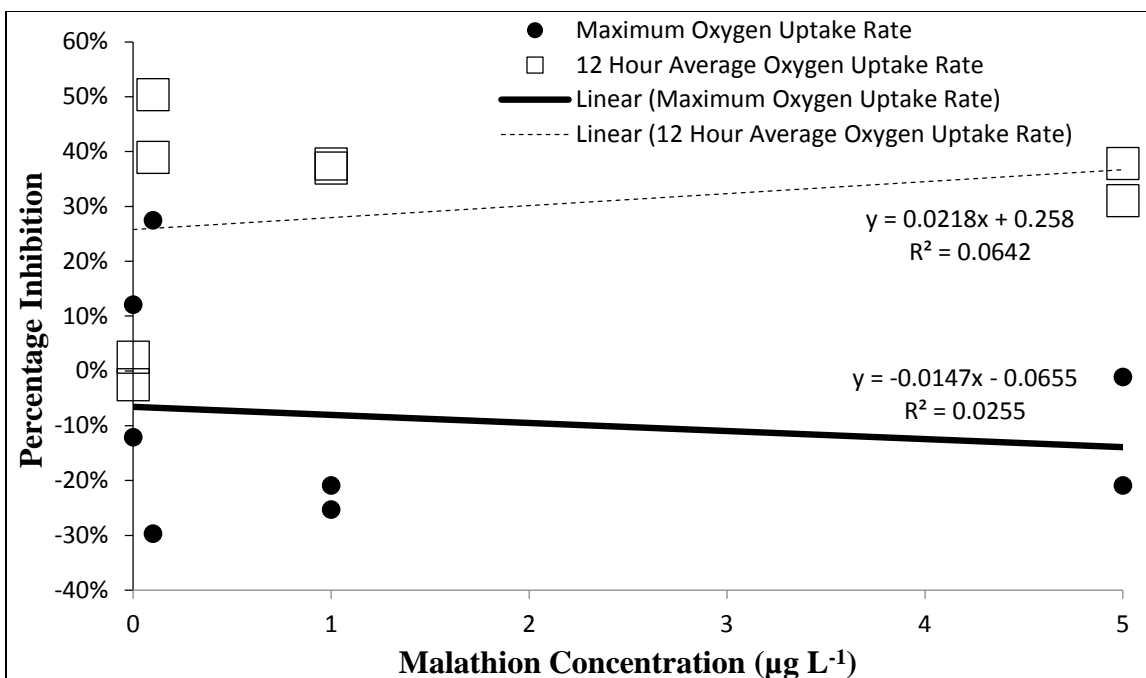


Figure 26: Respirometry Test 2 – Percentage Inhibition

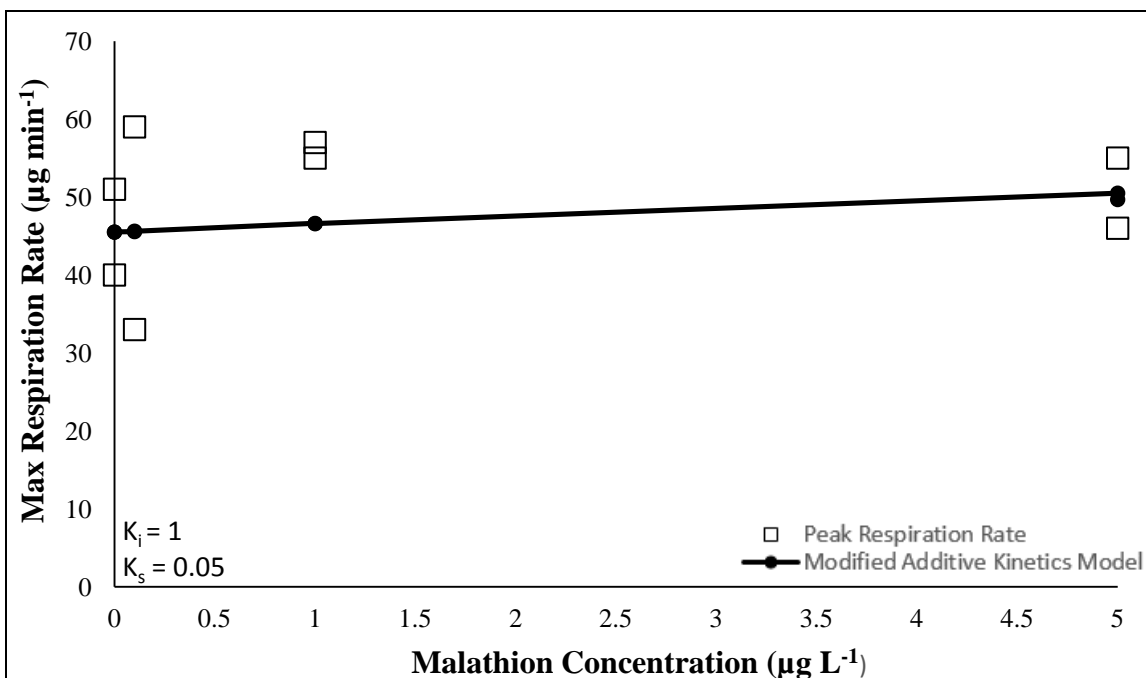


Figure 27: Respirometry Test 2 – Additive Kinetics Model

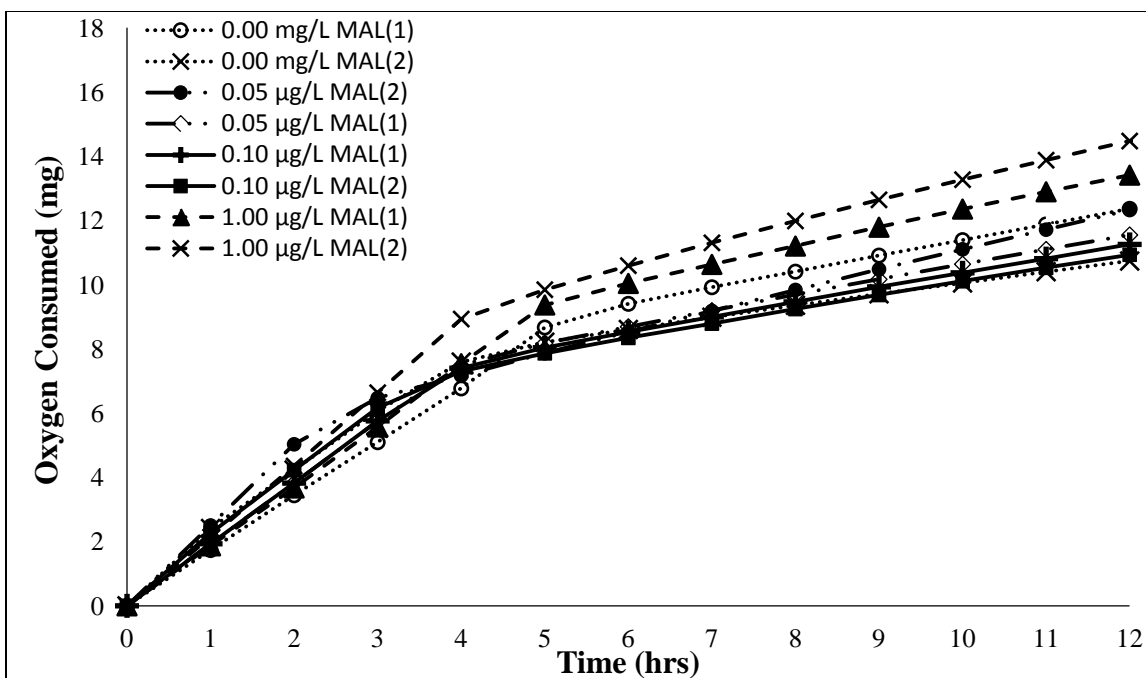


Figure 28: Respirometry Test 3 – Cumulative Oxygen Consumption

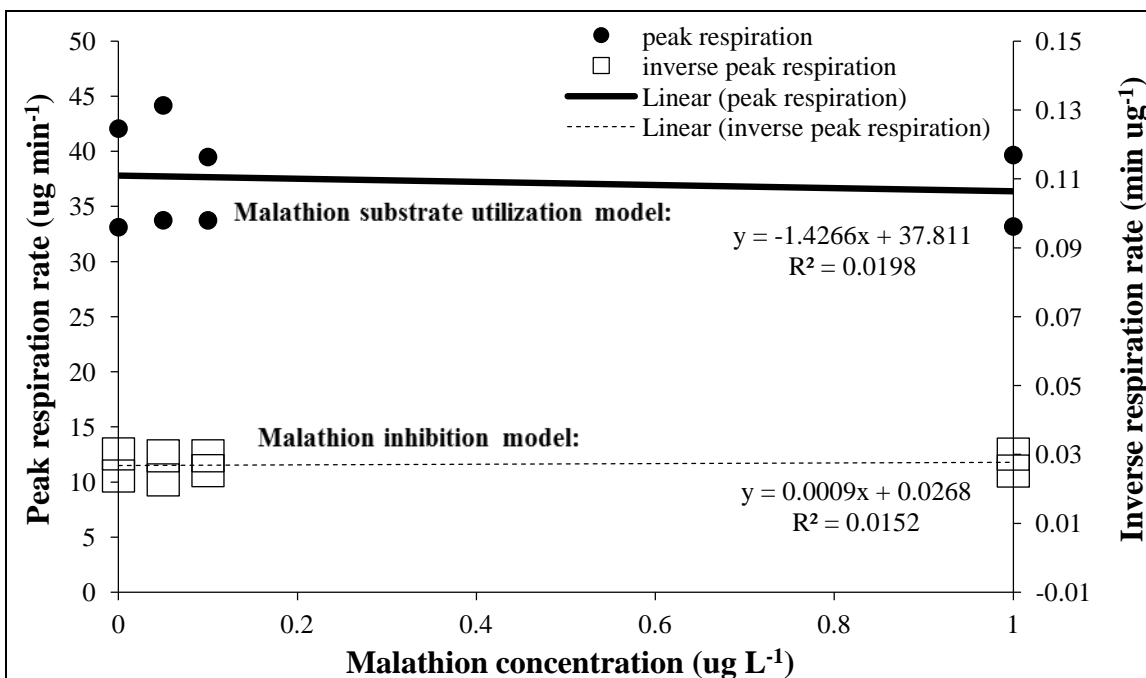


Figure 29: Respirometry Test 3 – Substrate Utilization & Inhibition Linearization Plots

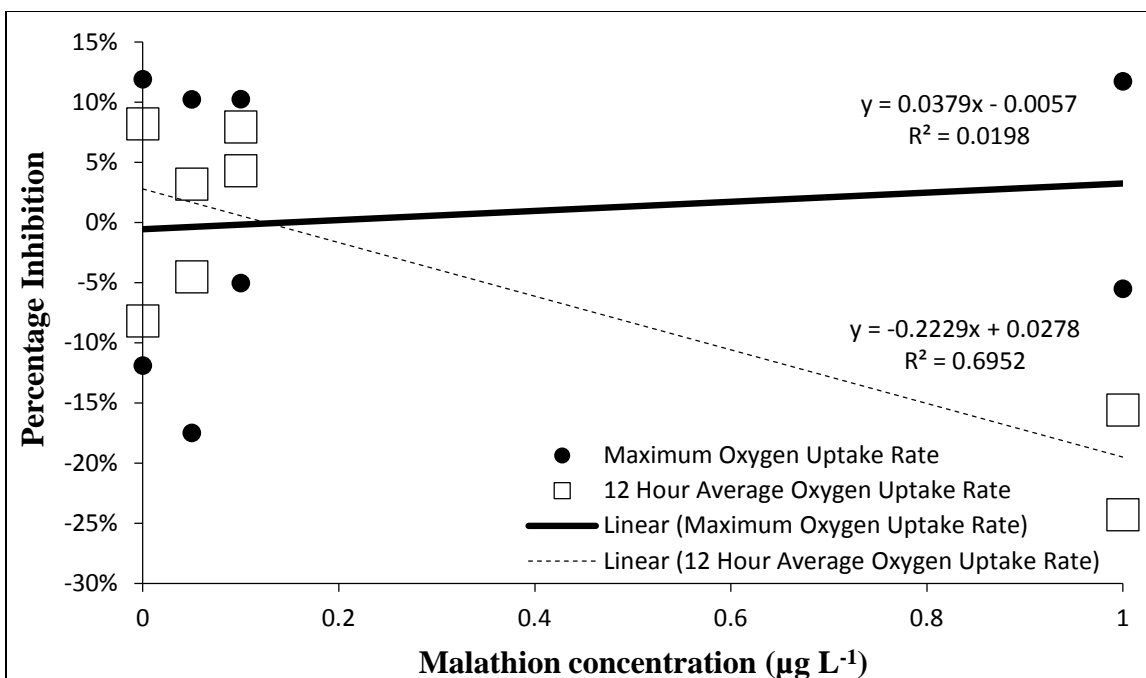


Figure 30: Respirometry Test 3 – Percentage Inhibition

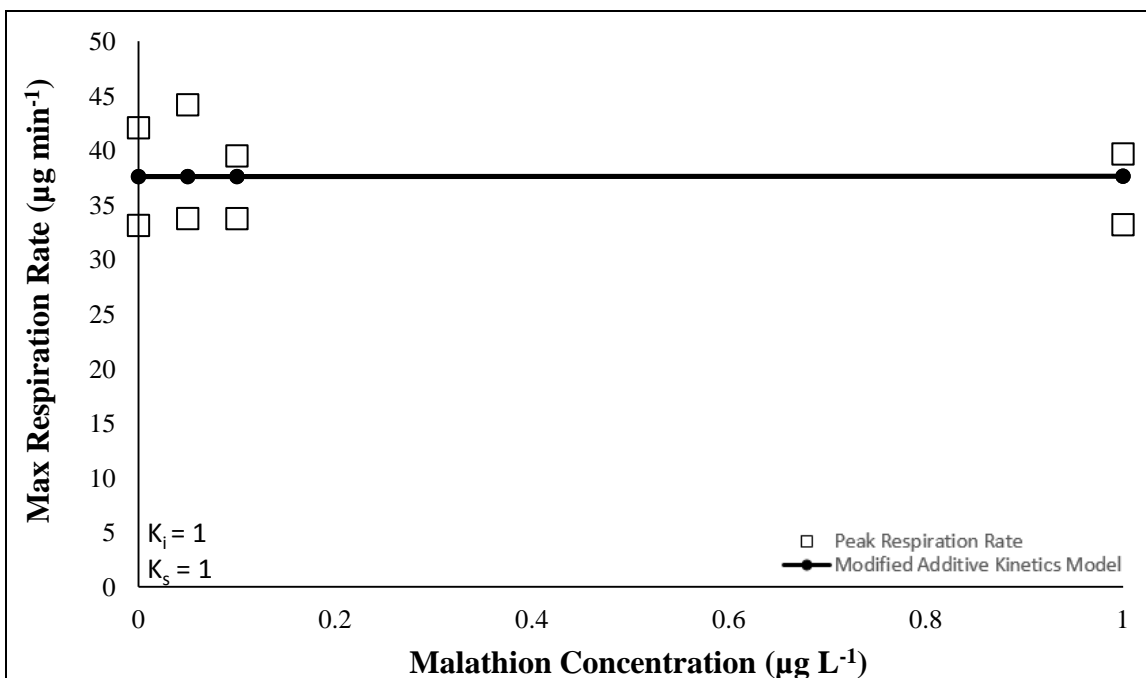


Figure 31: Respirometry Test 3 – Additive Kinetics Model

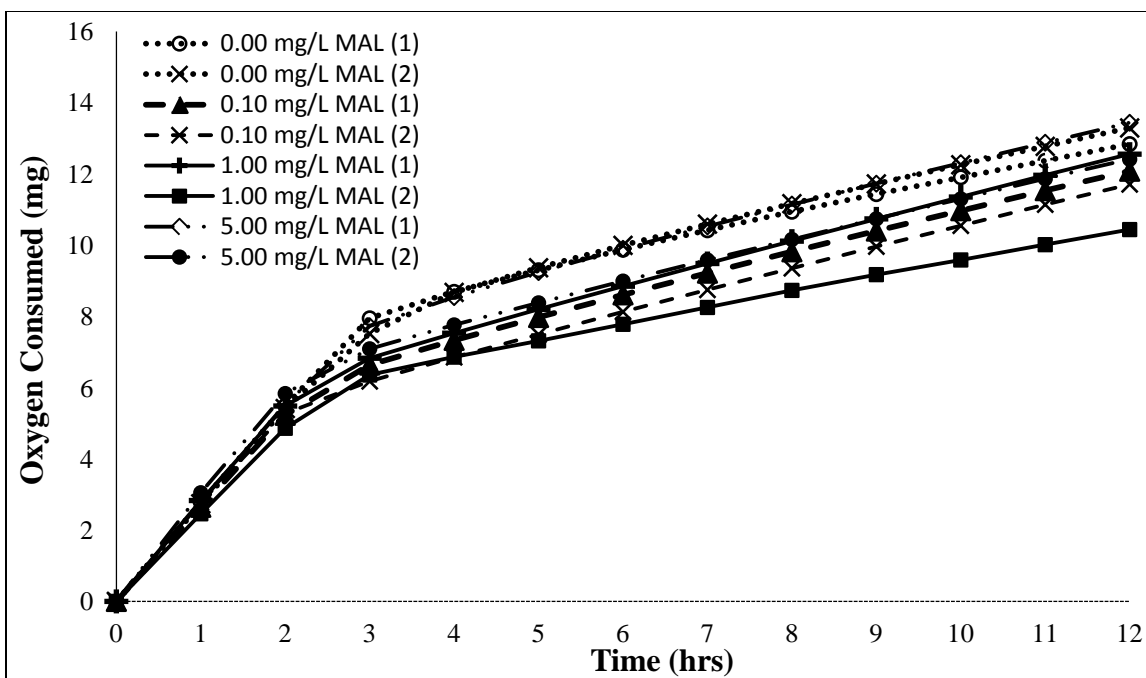


Figure 32: Respirometry Test 4 – Cumulative Oxygen Consumption

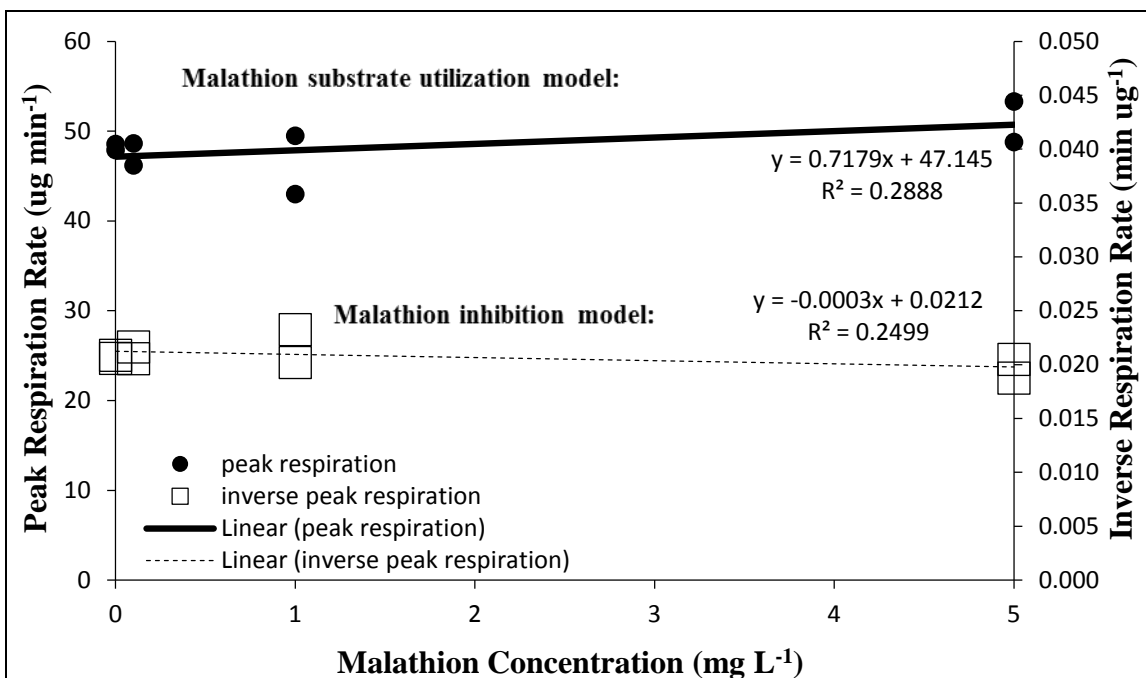


Figure 33: Respirometry Test 4 – Substrate Utilization & Inhibition Linearization Plots

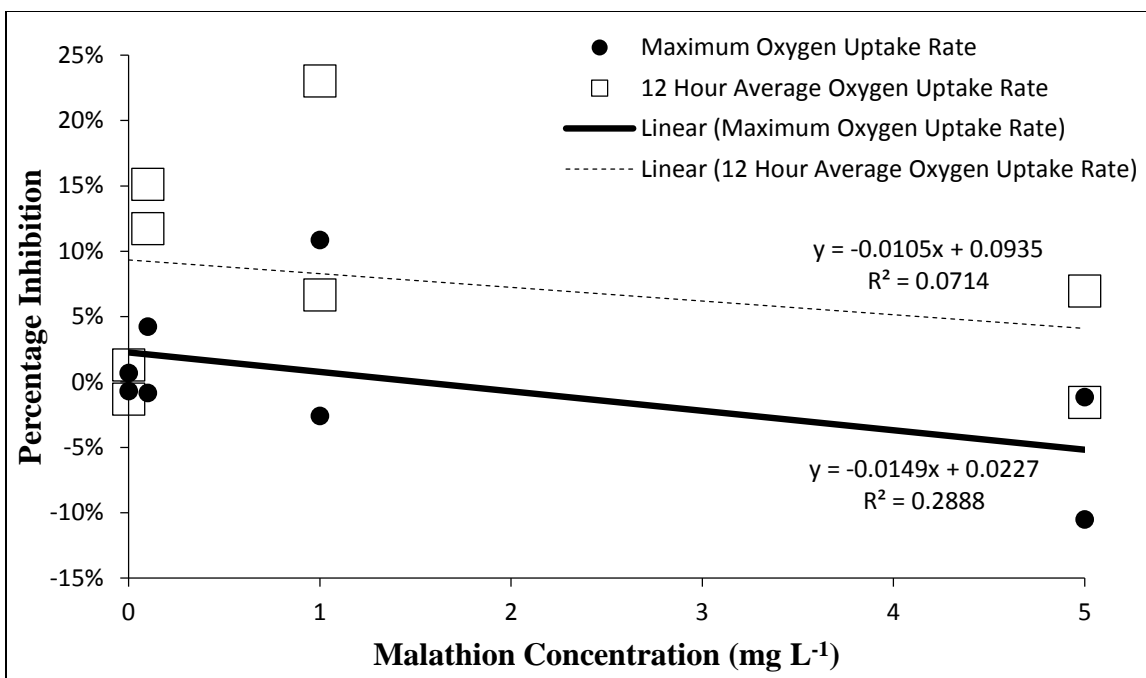


Figure 34: Respirometry Test 4 – Percentage Inhibition

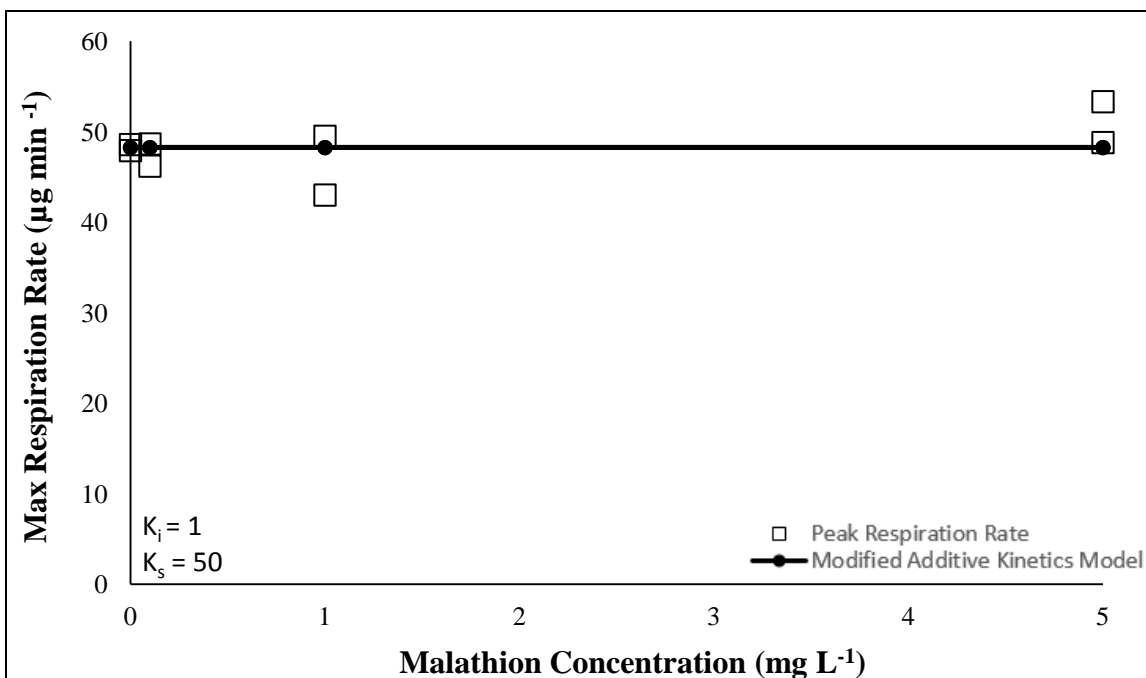


Figure 35: Respirometry Test 4 – Additive Kinetics Model

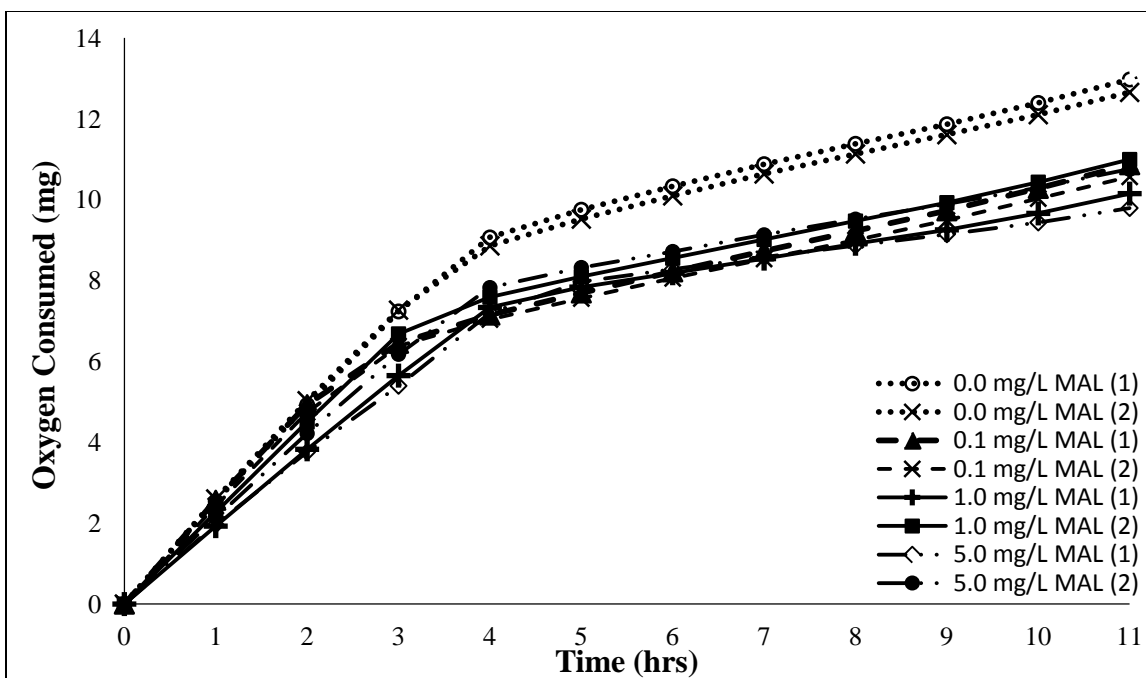


Figure 36: Respirometry Test 5 – Cumulative Oxygen Consumption

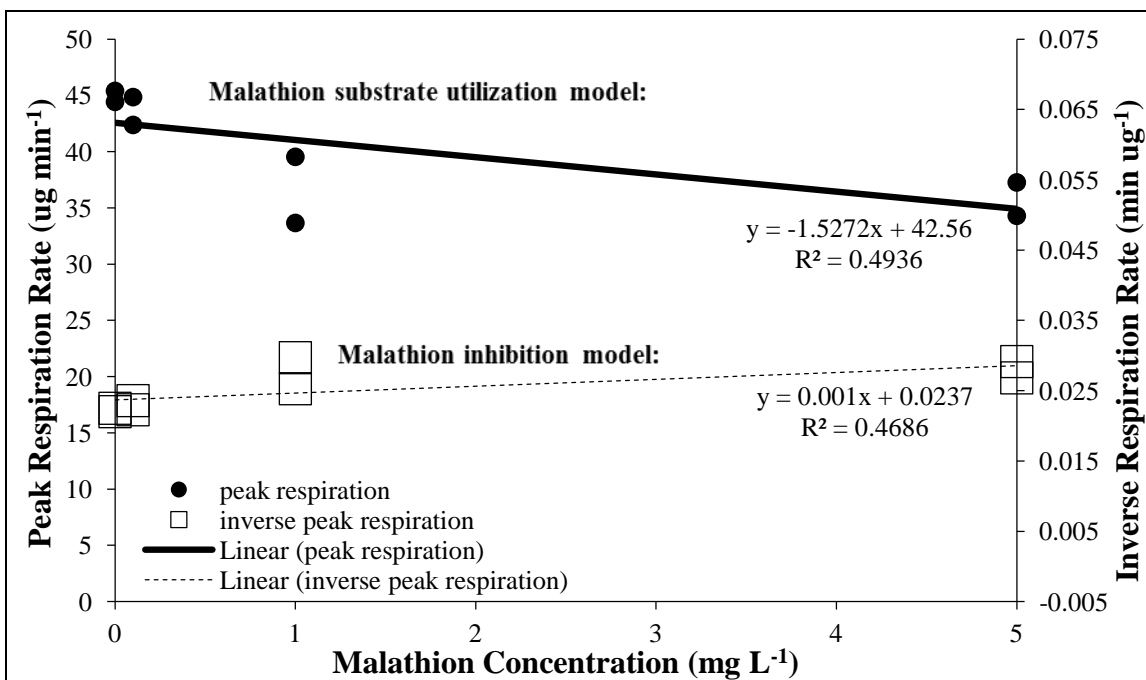


Figure 37: Respirometry Test 5 – Substrate Utilization & Inhibition Linearization Plots

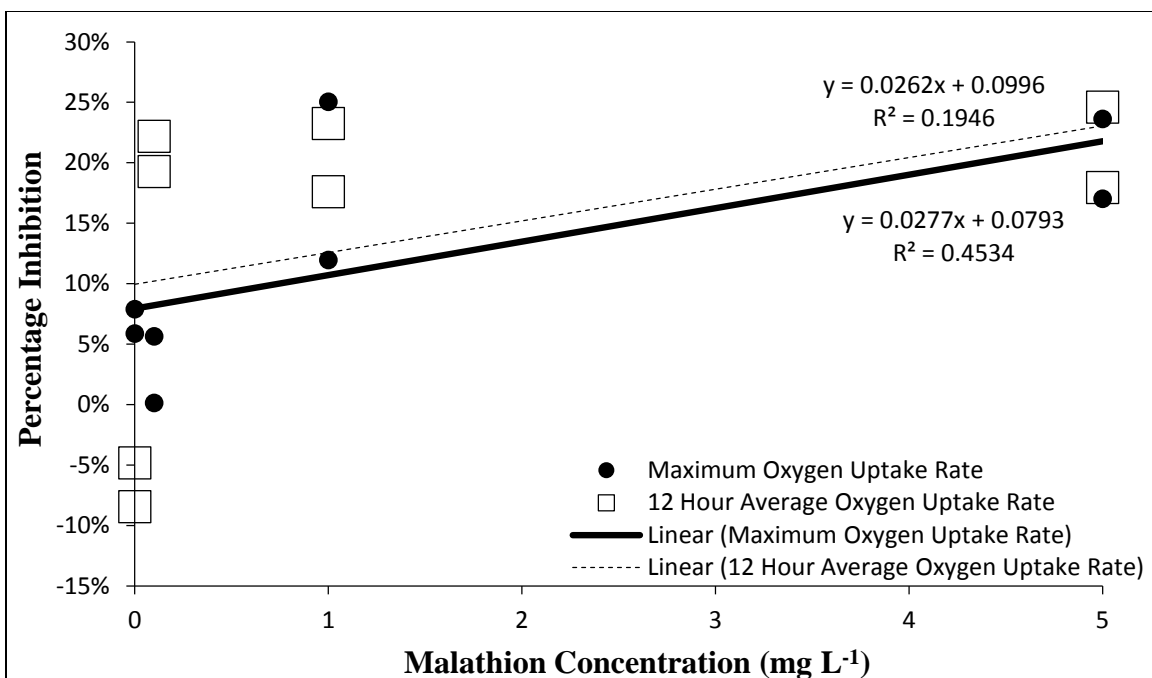


Figure 38: Respirometry Test 5 – Percentage Inhibition

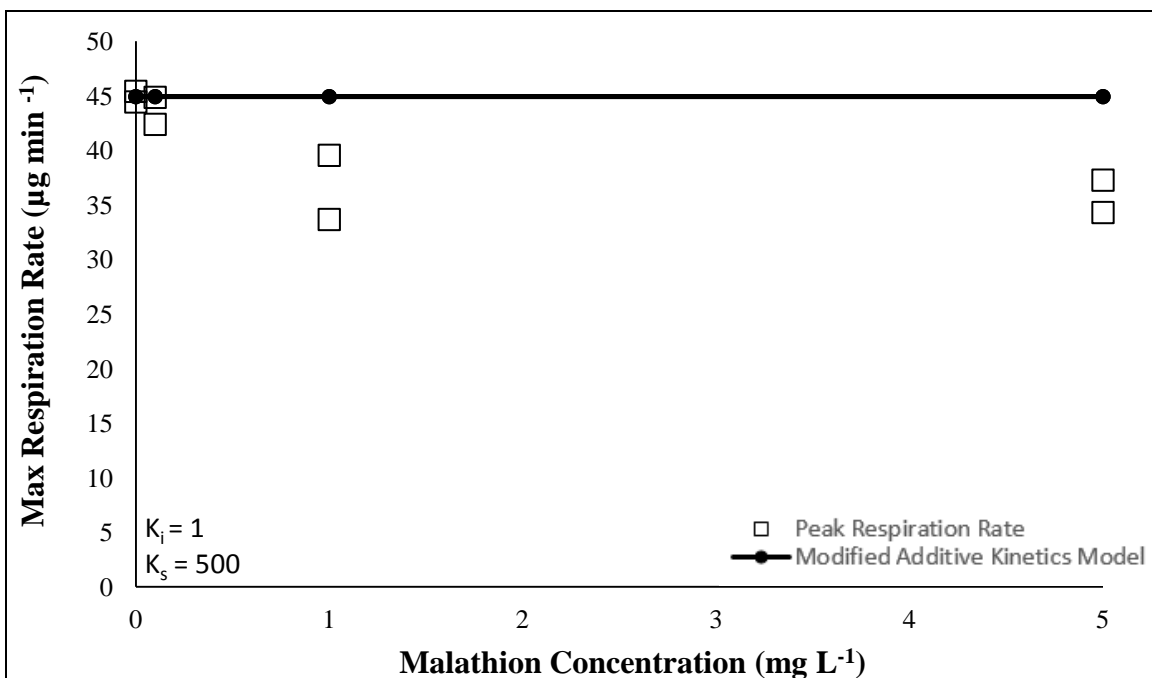


Figure 39: Respirometry Test 5 – Additive Kinetics Model



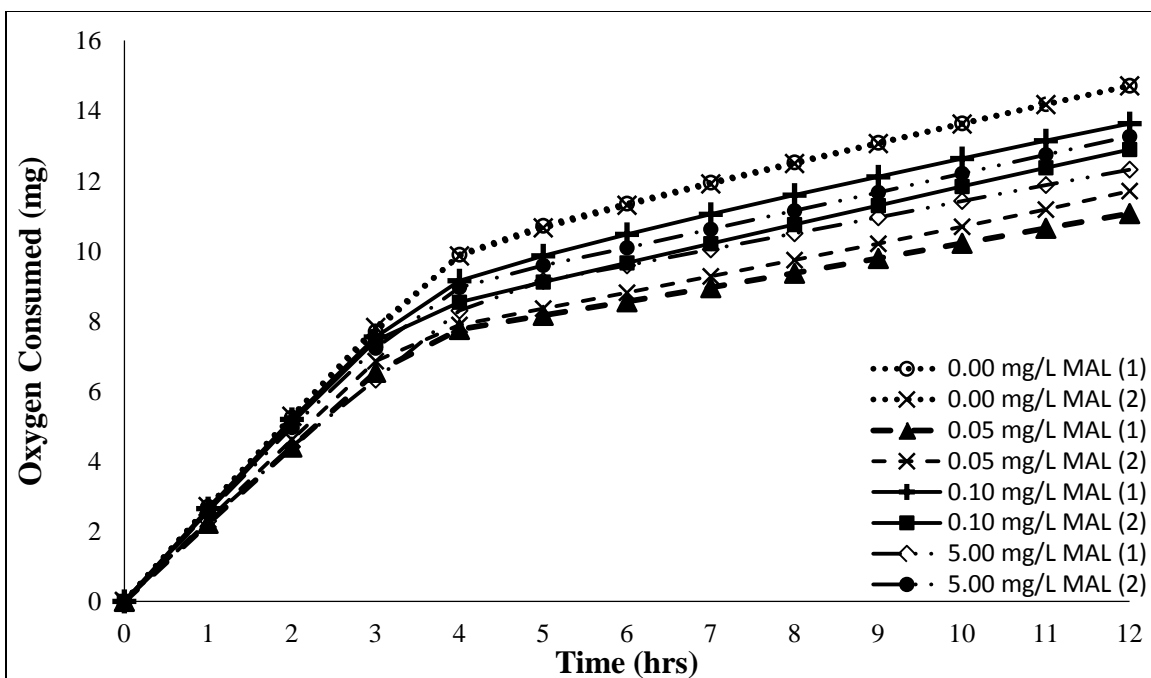


Figure 40: Respirometry Test 6 – Cumulative Oxygen Consumption

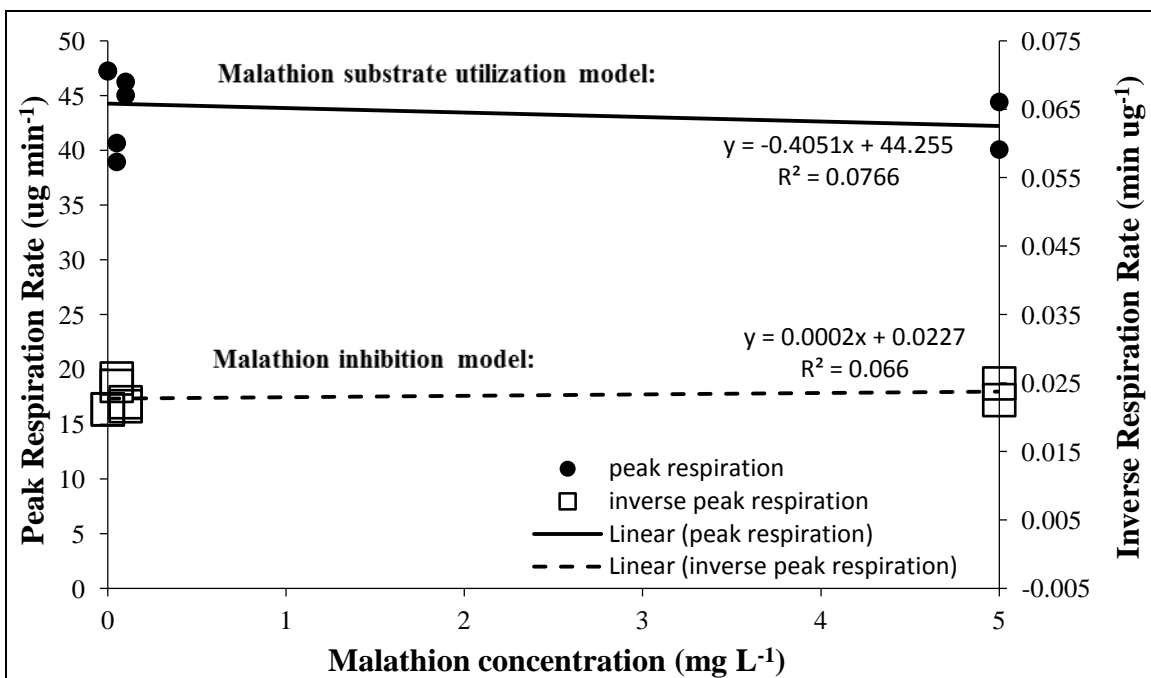


Figure 41: Respirometry Test 6 – Substrate Utilization & Inhibition Linearization Plots

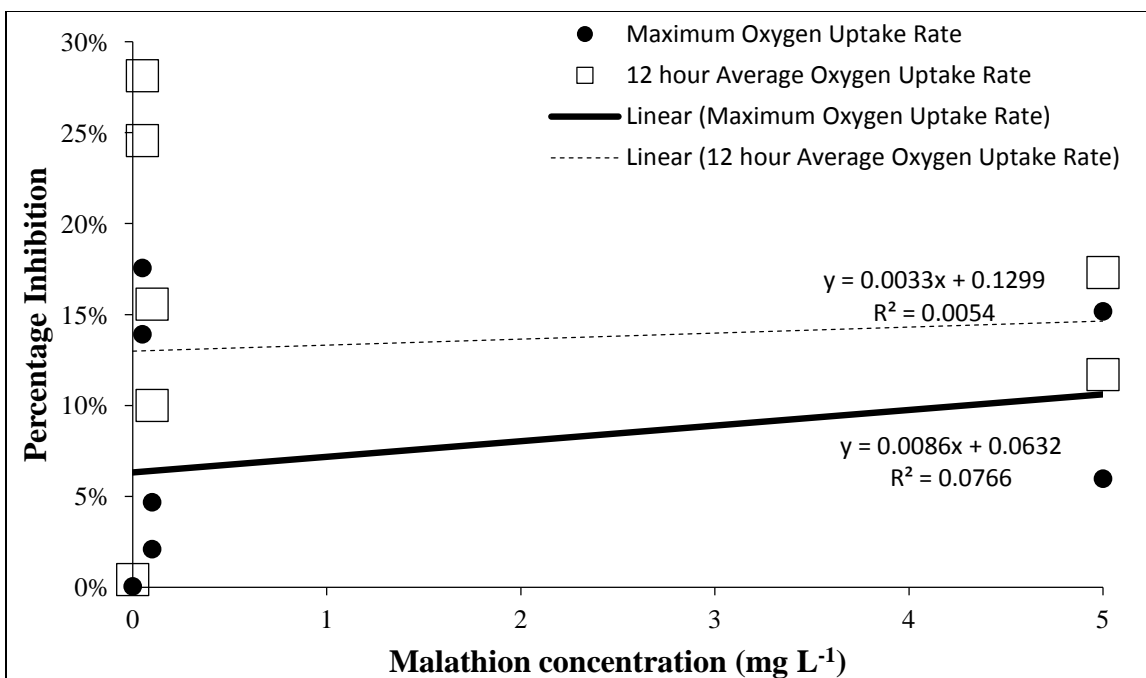


Figure 42: Respirometry Test 6 – Percentage Inhibition

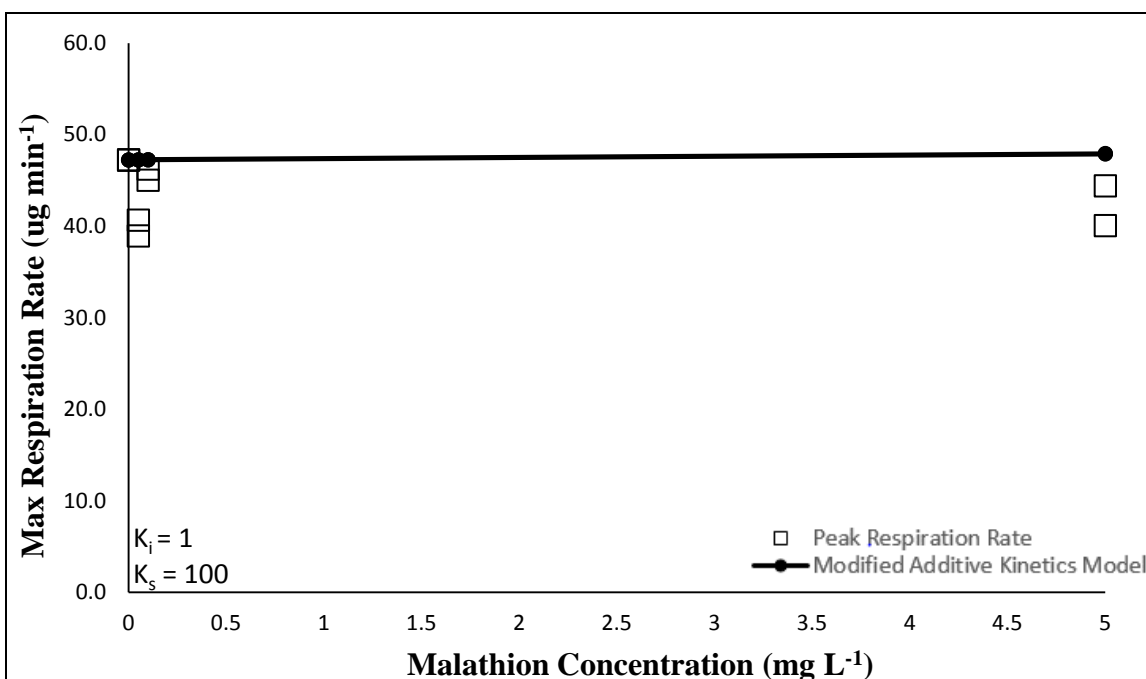


Figure 43: Respirometry Test 6 – Additive Kinetics Model

## Appendix C: Batch Test Performance Data

**Table 10: Batch Test COD Removal Performance**

Malathion Concentration (mg L <sup>-1</sup> )	Batch Test	Total Reduction (%)	Peak Utilization Rate (mg hr <sup>-1</sup> )	Peak Utilization Time (hrs)	Final COD Concentration (mg L <sup>-1</sup> )	TSS (mg L <sup>-1</sup> )
0.00	1	79.1	34.18	0 – 2	20.46	1440.0
	2	85.3	23.82	0 – 2	12.53	1125.5
	3	74.6	34.47	0 – 2	28.07	663.7
0.10	1	81.8	19.40	0 – 2	11.49	1317.7
	2	74.7	18.27	0 – 2	18.92	1107.3
	3	79.7	28.80	0 – 2	21.21	654
3.00	1	79.8	12.70	0 – 2	11.49	1354.5
	2	56.7	20.75	0 – 2	53.64	1054.3
	3	78.7	29.79	0 – 2	21.72	688.2

**Table 11: Batch Test NH<sub>3</sub> Removal Performance**

Malathion Concentration (mg L <sup>-1</sup> )	Batch Test	Reduction (%)	Peak Utilization Rate (mg hr <sup>-1</sup> )	Peak Utilization Time (hrs)	Final NH <sub>3</sub> Concentration (mg L <sup>-1</sup> )	TSS (mg L <sup>-1</sup> )
0.00	1	100*	3.51	0 – 3	0.0*	1440.0
	2	100*	2.70	1 – 4	0.0*	1125.5
	3	71	1.40	2 – 9	0.0*	663.7
0.10	1	100*	3.46	0 – 3	0.0*	1317.7
	2	100*	3.63	1 – 4	0.0*	1107.3
	3	69	1.99	2 – 7	0.0*	654
3.00	1	100*	3.29	0 – 3	0.0*	1354.5
	2	100*	1.75	1 – 7	0.0*	1054.3
	3	70	1.24	2 – 10	0.0*	688.2

\* Quantities below detectable limits

**Table 12: Batch Test NO<sub>3</sub> Production Performance**

Malathion Concentration (mg L <sup>-1</sup> )	Batch Test	Increase (%)	Peak utilization rate (mg/hr)	Peak Utilization Time (hrs)	Final NO <sub>3</sub> Concentration (mg L <sup>-1</sup> )	TSS (mg L <sup>-1</sup> )
0.00	1	77.9	2.44	0 – 4	20.05	1440.0
	2	134.5	1.60	0 – 7	20.02	1125.5
	3	81.5	0.90	0 – 12	23.28	663.7
0.10	1	93.5	2.52	0 – 4	20.82	1317.7
	2	127.2	1.71	0 – 7	20.91	1107.3
	3	85.9	0.98	0 – 12	23.45	654
3.00	1	80.9	2.41	0 – 4	19.39	1354.5
	2	143.9	1.46	0 – 7	19.61	1054.3
	3	83.1	0.87	0 – 12	22.71	688.2

## Appendix D: Batch Test 1 Results

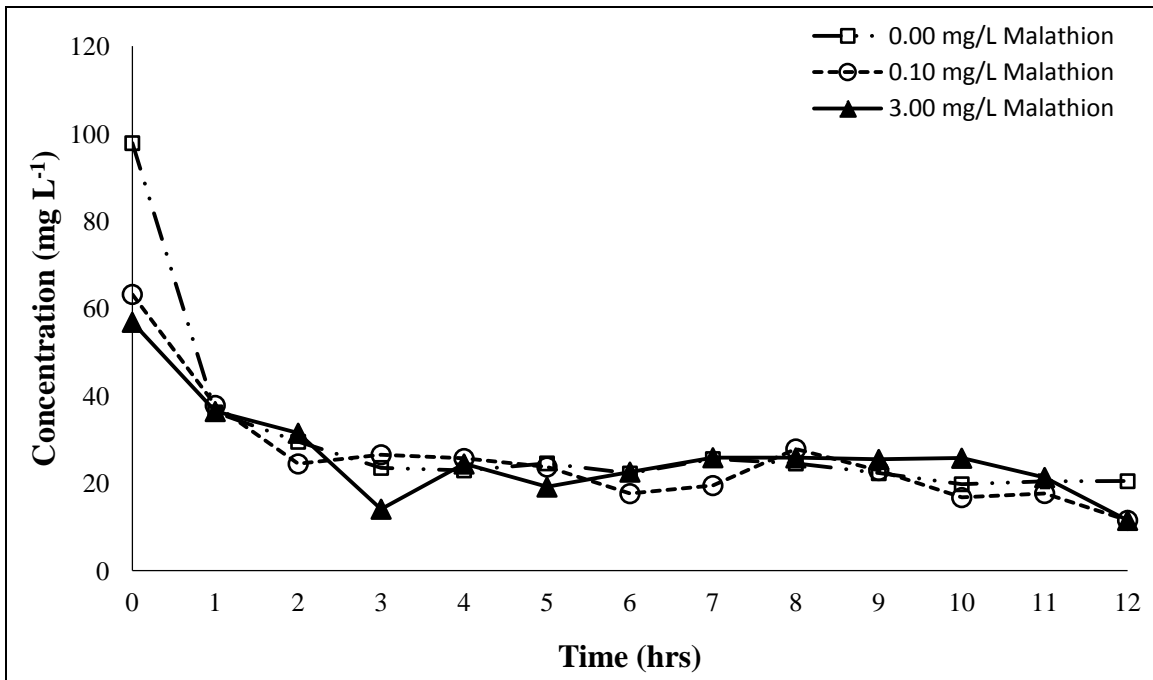


Figure 44: Batch Test 1 – COD Removal

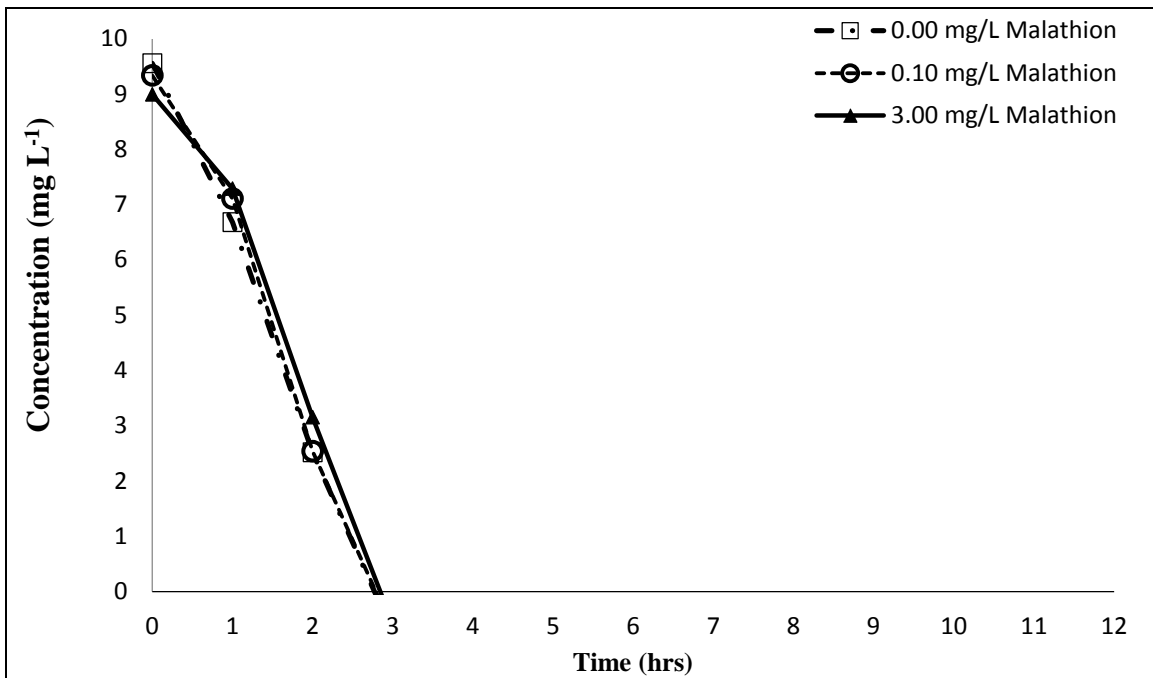


Figure 45: Batch Test 1 – NH<sub>3</sub> Removal

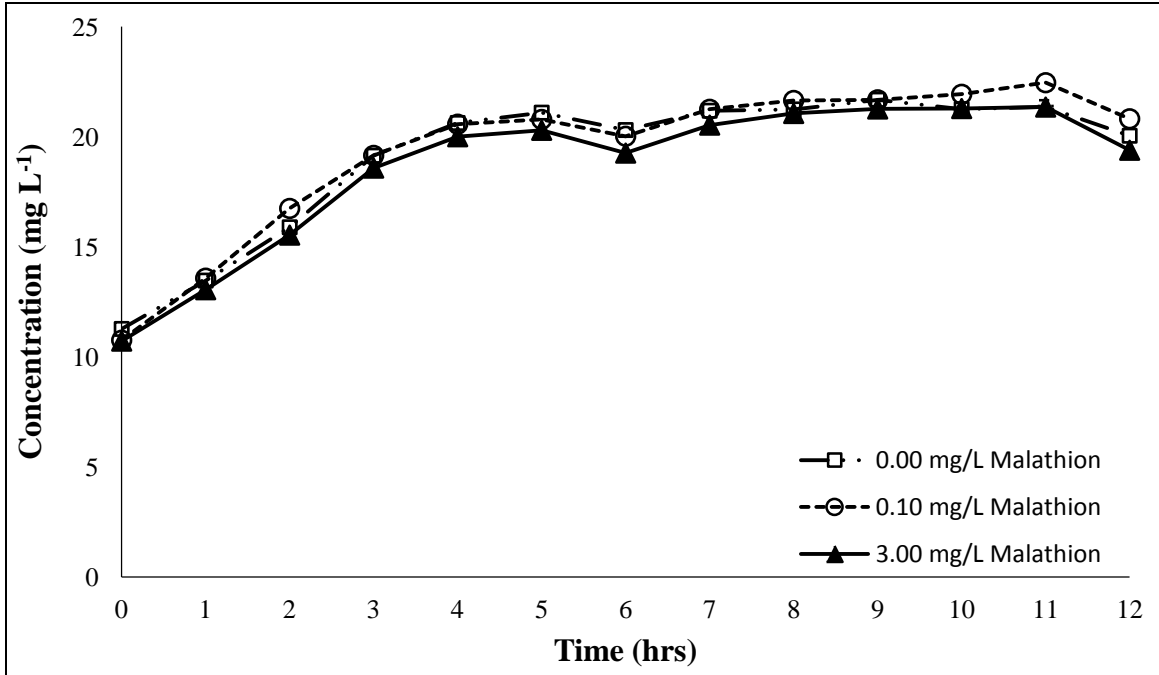


Figure 46: Batch Test 1 –  $\text{NO}_3$  Production

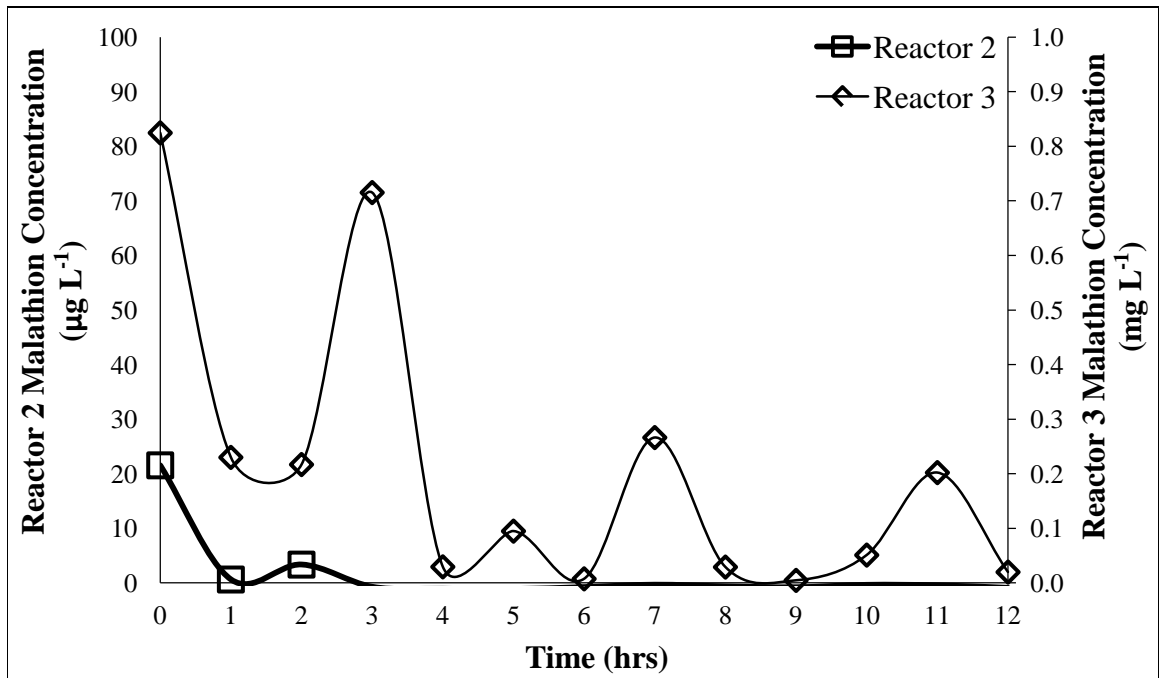
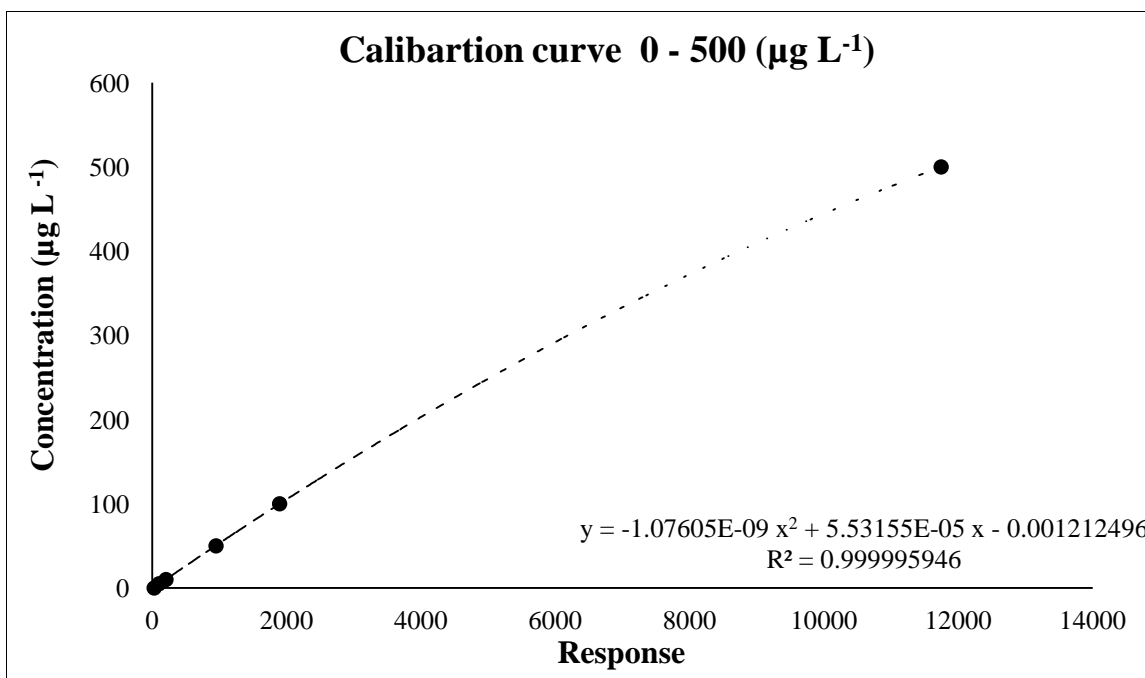
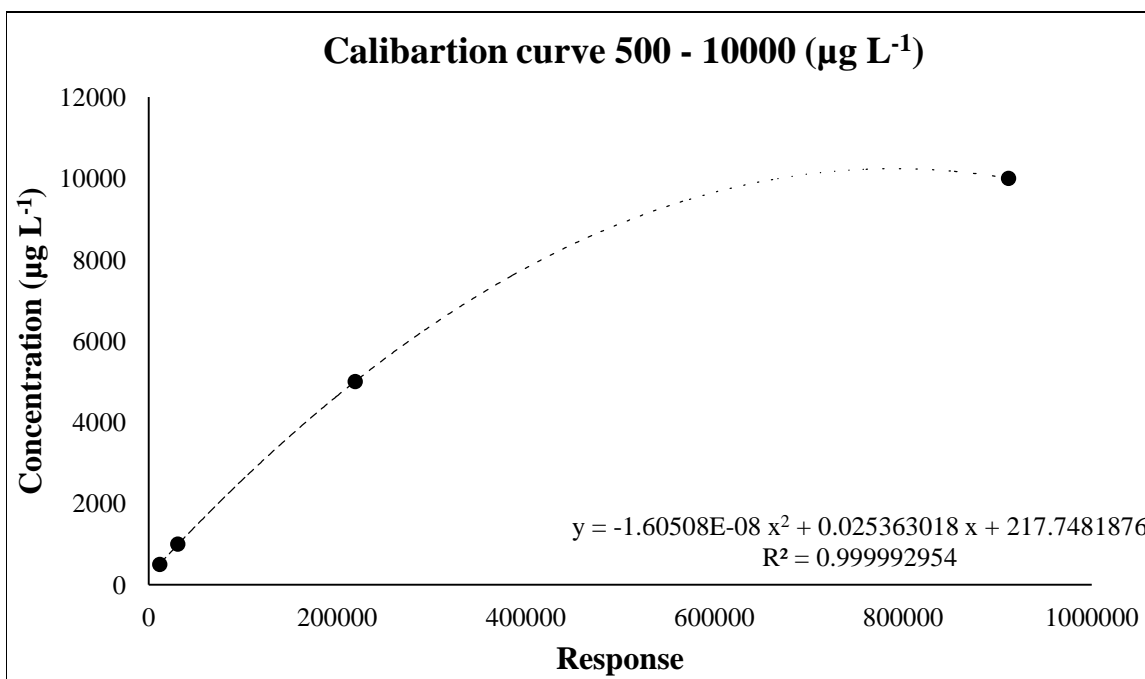


Figure 47: Batch Test 1 – Malathion Degradation

Table 13: Batch Test 1 – GC/MS Data File Sample Reference								
File #	Concentration				File #	Reactor	Hour	Sample
1	MeOH Blank				50	2	7	2
2	MeOH Blank				51	2	7	2
3	0.001 mg L <sup>-1</sup>				52	2	6	1
4	0.001 mg L <sup>-1</sup>				53	2	6	2
5	0.005 mg L <sup>-1</sup>				54	2	5	1
6	0.005 mg L <sup>-1</sup>				55	2	5	2
7	0.010 mg L <sup>-1</sup>				56	2	4	1
8	0.010 mg L <sup>-1</sup>				57	2	4	2
9	0.050 mg L <sup>-1</sup>				58	2	3	1
10	0.050 mg L <sup>-1</sup>				59	2	3	2
11	0.100 mg L <sup>-1</sup>				60	MeOH Blank		
12	0.100 mg L <sup>-1</sup>				61	1.000 mg L <sup>-1</sup>		
13	0.500 mg L <sup>-1</sup>				62	MeOH Blank		
14	0.500 mg L <sup>-1</sup>				63	2	2	1
15	1.000 mg L <sup>-1</sup>				64	2	2	2
16	1.000 mg L <sup>-1</sup>				65	2	1	1
17	5.000 mg L <sup>-1</sup>				66	2	1	2
18	5.000 mg L <sup>-1</sup>				67	2	0	1
19	10.00 mg L <sup>-1</sup>				68	2	0	2
20	10.00 mg L <sup>-1</sup>				69	MeOH Blank		
21	MeOH Blank				70	3	12	1
22	H2O Blank				71	3	12	2
23	MeOH Blank				72	3	11	1
24	1.000 mg L <sup>-1</sup>				73	3	11	2
25	1.000 mg L <sup>-1</sup>				74	3	10	1
26	MeOH Blank				75	3	10	2
27	MeOH Blank				76	3	9	1
28	MeOH Blank				77	3	9	2
	Reactor	Hour	Sample		78	3	8	1
29	1	12	1		79	3	8	2
30	1	12	2		80	3	7	1
31	1	8	1		81	3	7	2
32	1	8	2		82	MeOH Blank		
33	1	4	1		83	1.000 mg L <sup>-1</sup>		
34	1	4	2		84	MeOH Blank		
35	1	0	1		85	3	6	1
36	1	0	2		86	3	6	2
37	MeOH Blank				87	3	5	1
38	1.000 mg L <sup>-1</sup>				88	3	5	2
39	MeOH Blank				89	3	4	1
40	2	12	1		90	3	4	2
41	2	12	2		91	3	3	1
41	2	11	1		92	3	3	2
43	2	11	2		93	3	2	1
44	2	10	1		94	3	2	2
45	2	10	2		95	3	1	1
46	2	9	1		96	3	1	2
47	2	9	2		97	3	0	1
48	2	8	1	98	3	0	2	
49	2	8	2					



**Figure 48: Batch Test 1 – Low Range Calibration Curve**



**Figure 49: Batch Test 1 – High Range Calibration Curve**

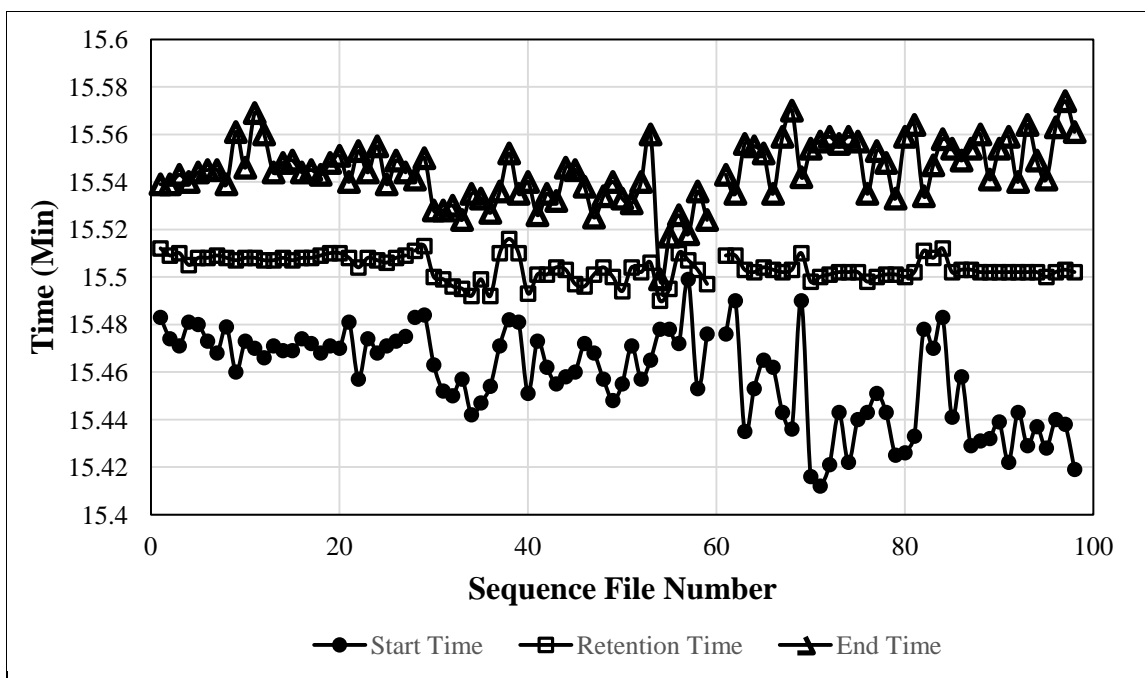


Figure 50: Batch Test 1 – GC/MS Retention Time Information

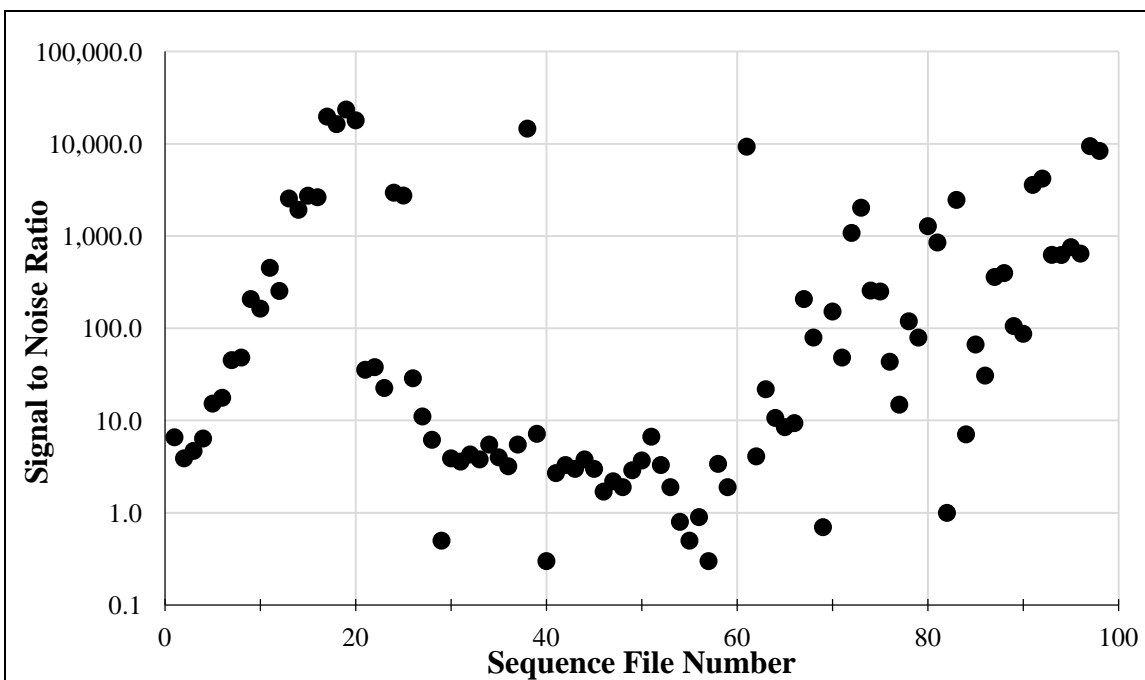


Figure 51: Batch Test 1 – Signal to Noise Ratio



## Appendix E: Batch Test 2 Results

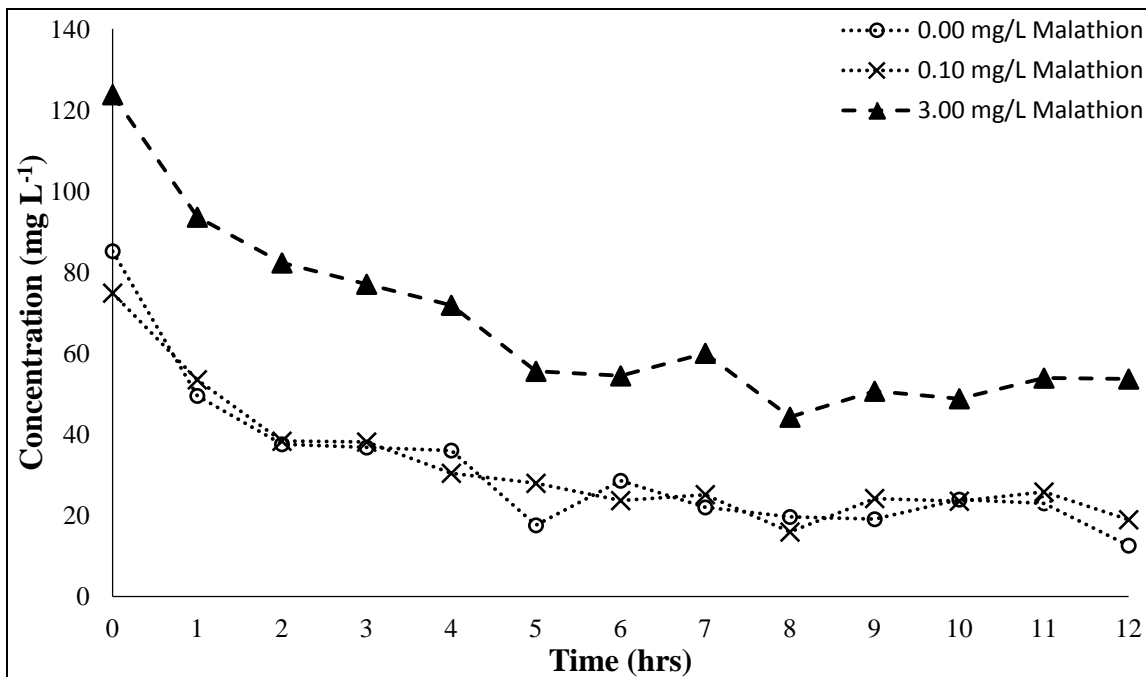


Figure 52: Batch Test 2 – COD Removal

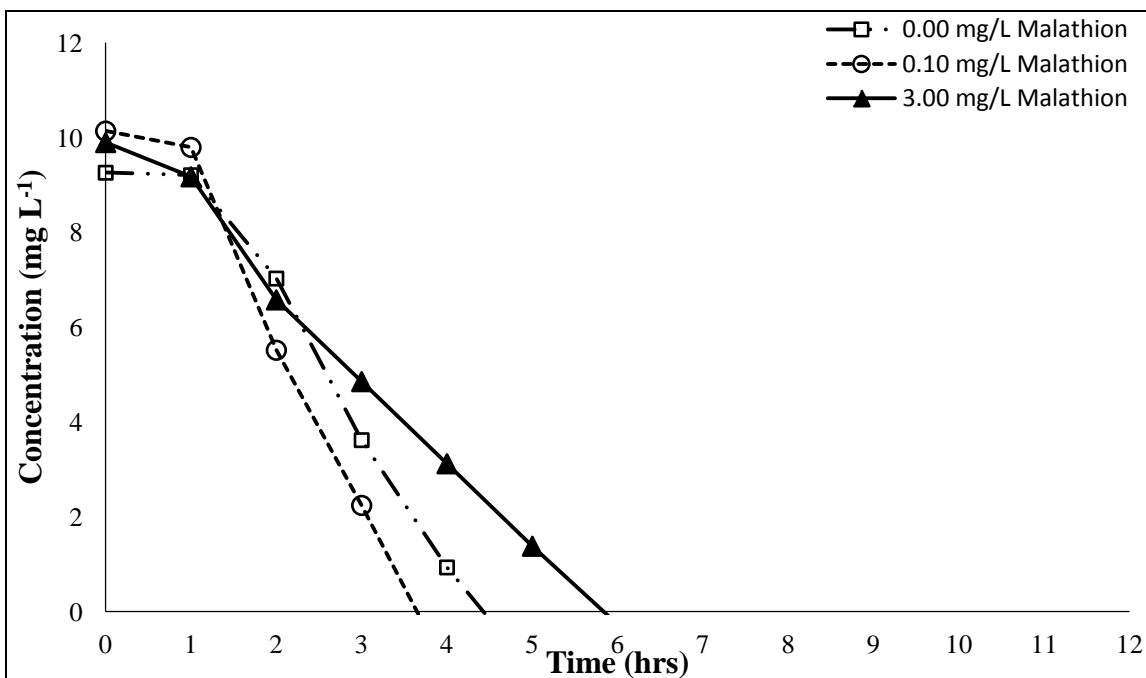


Figure 53: Batch Test 2 – NH<sub>3</sub> Removal

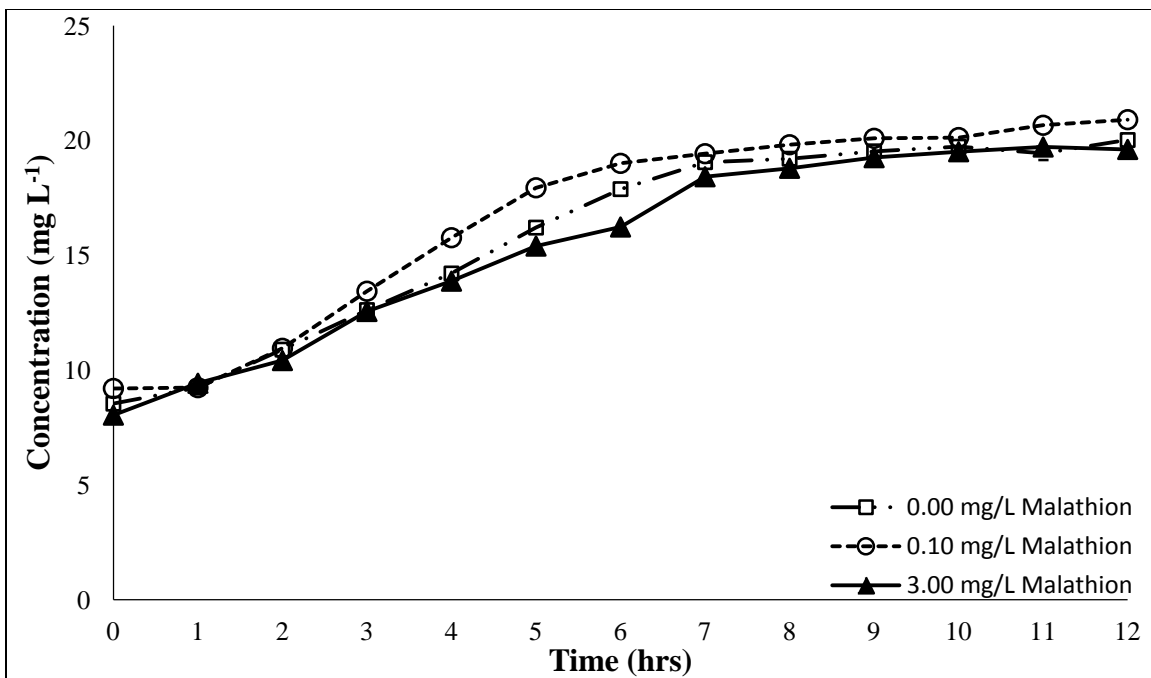


Figure 54: Batch Test 2 –  $\text{NO}_3$  Production

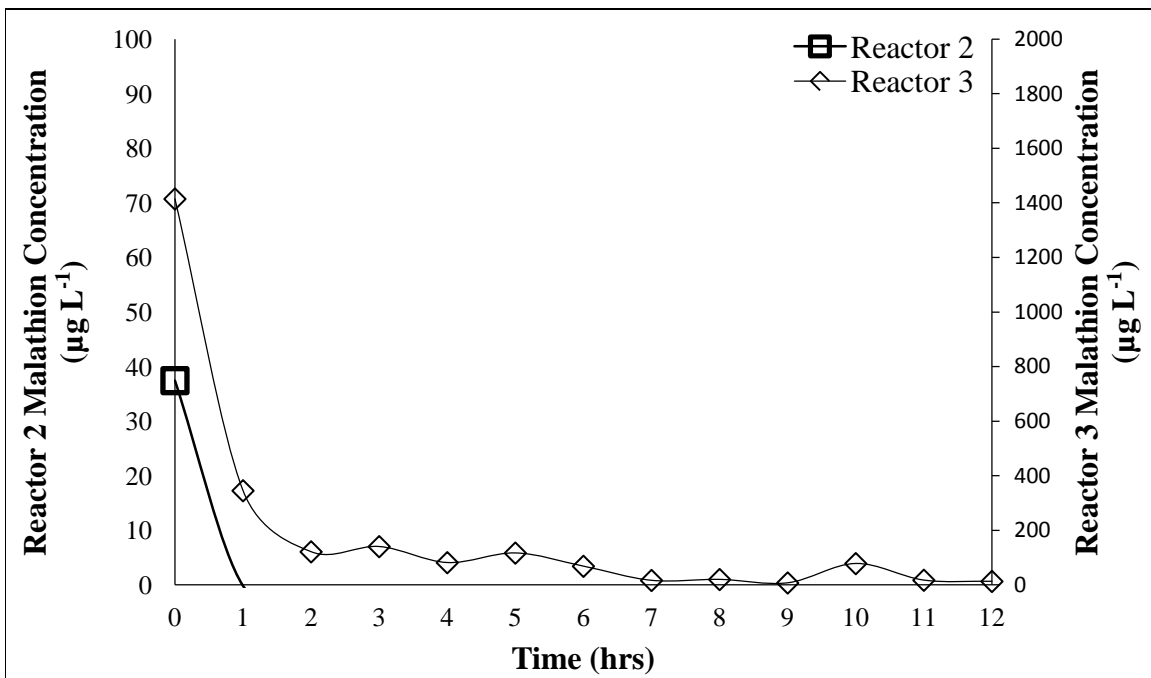
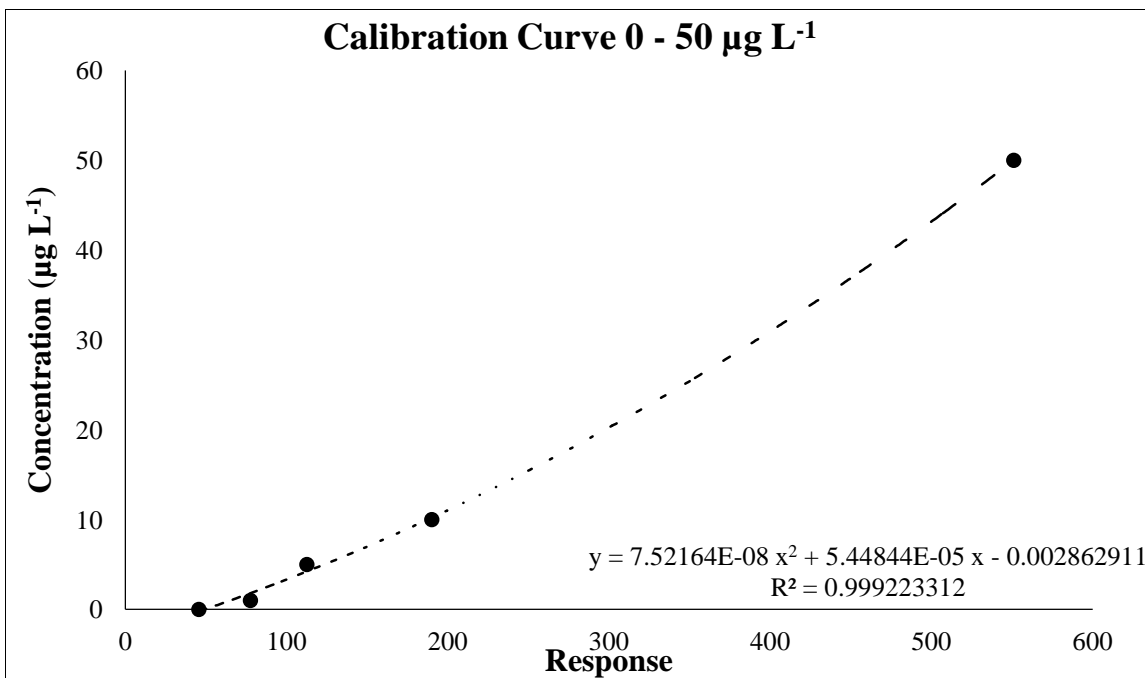


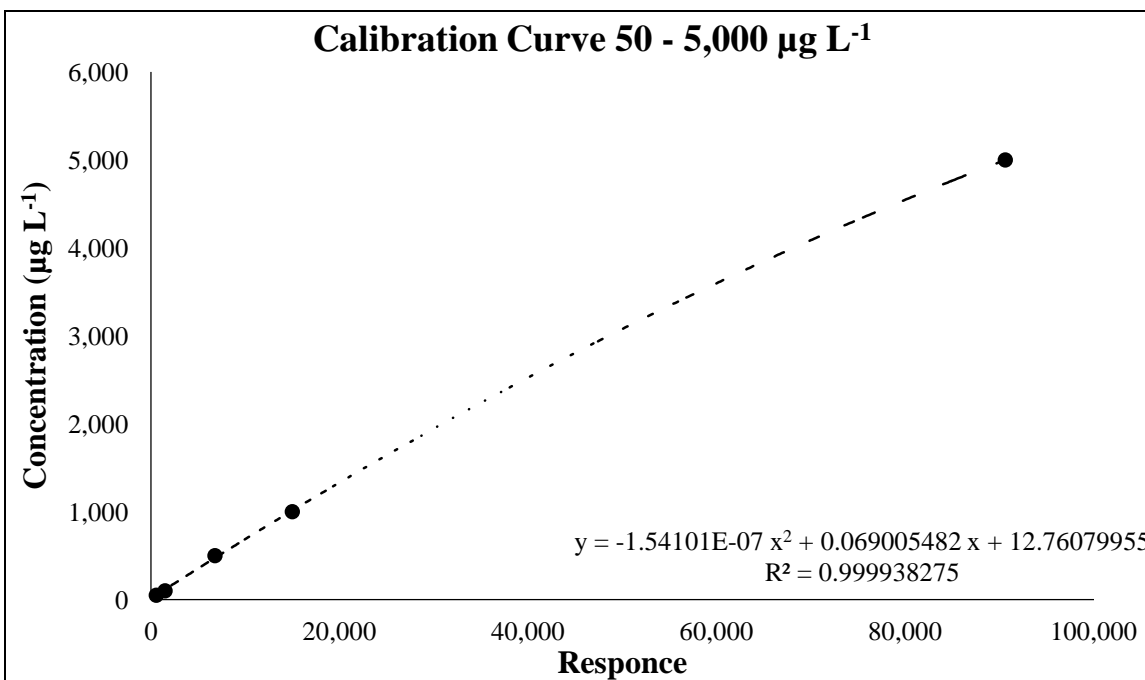
Figure 55: Batch Test 2 – Malathion Degradation

Table 14: Batch Test 2 – GC/MS Sequence Data File Reference																															
File #				Concentration				File #				Reactor				Hour				Sample											
1				MeOH Blank				50				2				7				2											
2				MeOH Blank				51				2				7				2											
3				0.001 mg L <sup>-1</sup>				52				2				6				1											
4				0.001 mg L <sup>-1</sup>				53				2				6				2											
5				0.005 mg L <sup>-1</sup>				54				2				5				1											
6				0.005 mg L <sup>-1</sup>				55				2				5				2											
7				0.010 mg L <sup>-1</sup>				56				2				4				1											
8				0.010 mg L <sup>-1</sup>				57				2				4				2											
9				0.050 mg L <sup>-1</sup>				58				2				3				1											
10				0.050 mg L <sup>-1</sup>				59				2				3				2											
11				0.100 mg L <sup>-1</sup>				60				MeOH Blank				100				MeOH Blank											
12				0.100 mg L <sup>-1</sup>				61				1.0 mg L <sup>-1</sup> Check Sample				101				1.0 mg L <sup>-1</sup> Check Sample											
13				0.500 mg L <sup>-1</sup>				62				MeOH Blank				102				MeOH Blank											
14				0.500 mg L <sup>-1</sup>				63				2				2				1											
15				1.000 mg L <sup>-1</sup>				64				2				2				2											
16				1.000 mg L <sup>-1</sup>				65				2				1				1											
17				5.000 mg L <sup>-1</sup>				66				2				1				2											
18				5.000 mg L <sup>-1</sup>				67				2				0				1											
19				10.00 mg L <sup>-1</sup>				68				2				0				2											
20				10.00 mg L <sup>-1</sup>				69				MeOH Blank				103				H <sub>2</sub> O Blank											
21				MeOH Blank				70				3				12				1											
22				H <sub>2</sub> O Blank				71				3				12				2											
23				MeOH Blank				72				3				11				1											
24				1.000 mg L <sup>-1</sup>				73				3				11				2											
25				1.000 mg L <sup>-1</sup>				74				3				10				1											
26				MeOH Blank				75				3				10				2											
27				MeOH Blank				76				3				9				1											
28				MeOH Blank				77				3				9				2											
				Reactor				Hour				Sample				78				3				8				1			
29				1				12				1				79				3				8				2			
30				1				12				2				80				3				7				1			
31				1				8				1				81				3				7				2			
32				1				8				2				82				MeOH Blank											
33				1				4				1				83				1.0 mg L <sup>-1</sup> Check Sample											
34				1				4				2				84				MeOH Blank											
35				1				0				1				85				3				6				1			
36				1				0				2				86				3				6				2			
37				MeOH Blank												87				3				5				1			
38				1.0 mg L <sup>-1</sup> Check Sample												88				3				5				2			
39				MeOH Blank												89				3				4				1			
40				2				12				1				90				3				4				2			
41				2				12				2				91				3				3				1			
41				2				11				1				92				3				3				2			
43				2				11				2				93				3				2				1			
44				2				10				1				94				3				2				2			
45				2				10				2				95				3				1				1			
46				2				9				1				96				3				1				2			
47				2				9				2				97				3				0				1			
48				2				8				1				98				3				0				2			
49				2				8				2				99				MeOH Blank											

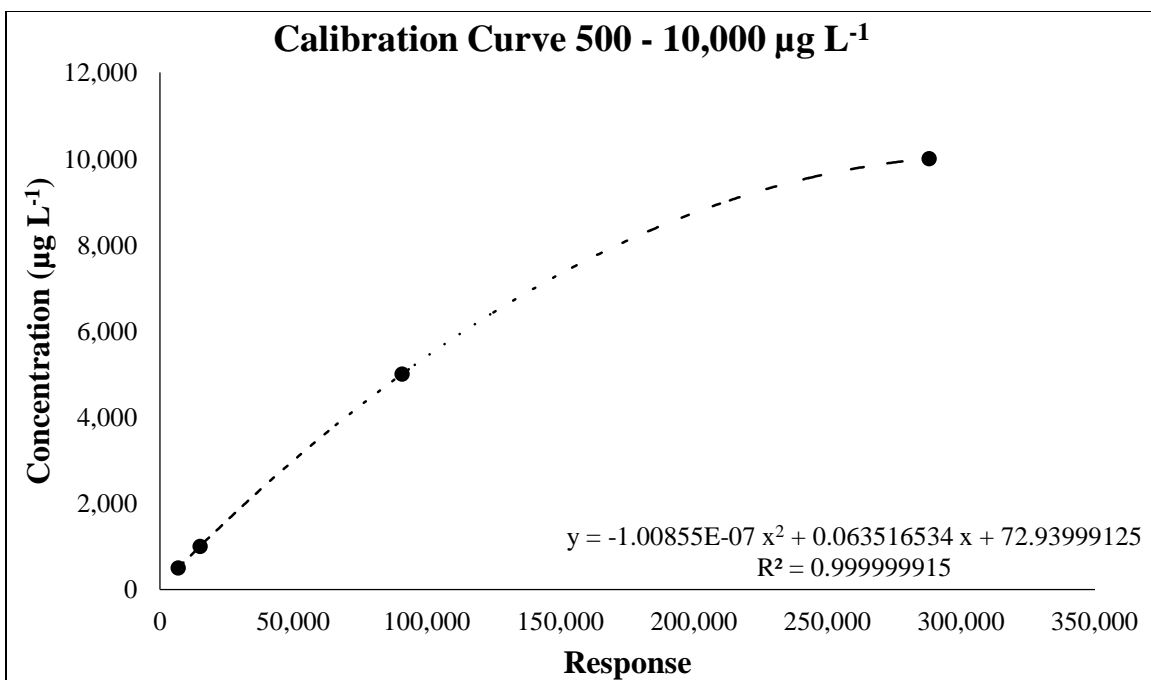
\* 2 Step dilution 100 mg L<sup>-1</sup> to 10 mg L<sup>-1</sup> then 10 mg L<sup>-1</sup> to 1 mg L<sup>-1</sup>



**Figure 56: Batch Test 2 – Low Range Calibration Curve**



**Figure 57: Batch Test 2 – Mid Range Calibration Curve**



**Figure 58: Batch Test 2 – High Range Calibration Curve**

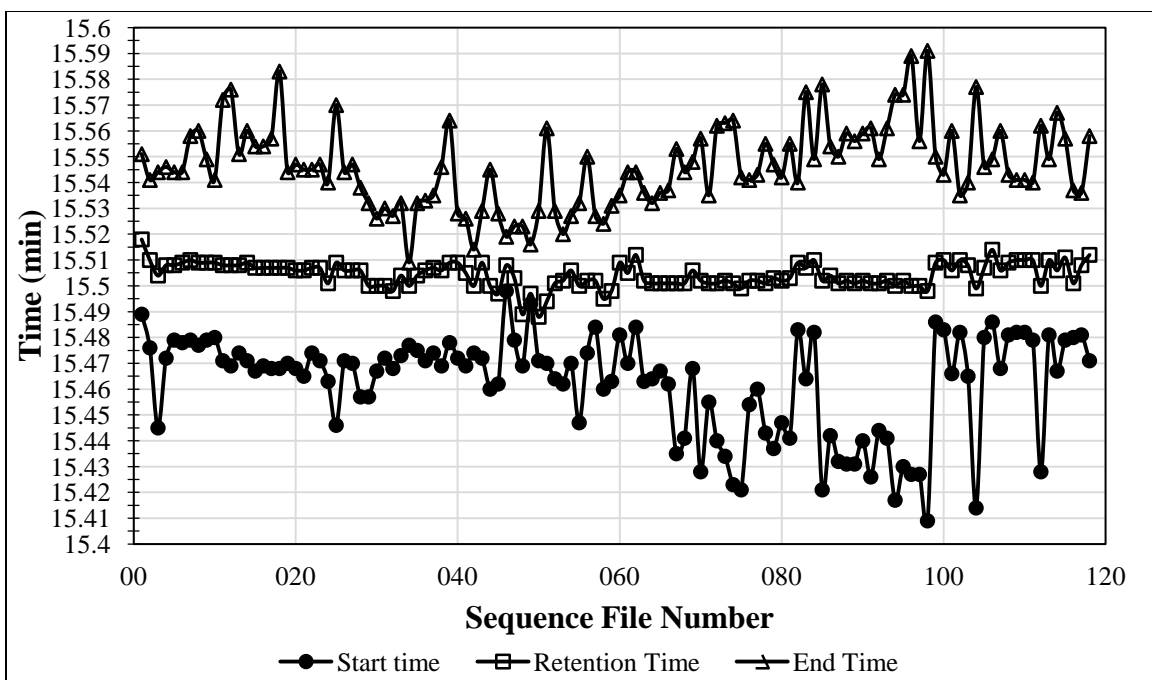


Figure 59: Batch Test 2 – GC/MS Retention Time Information

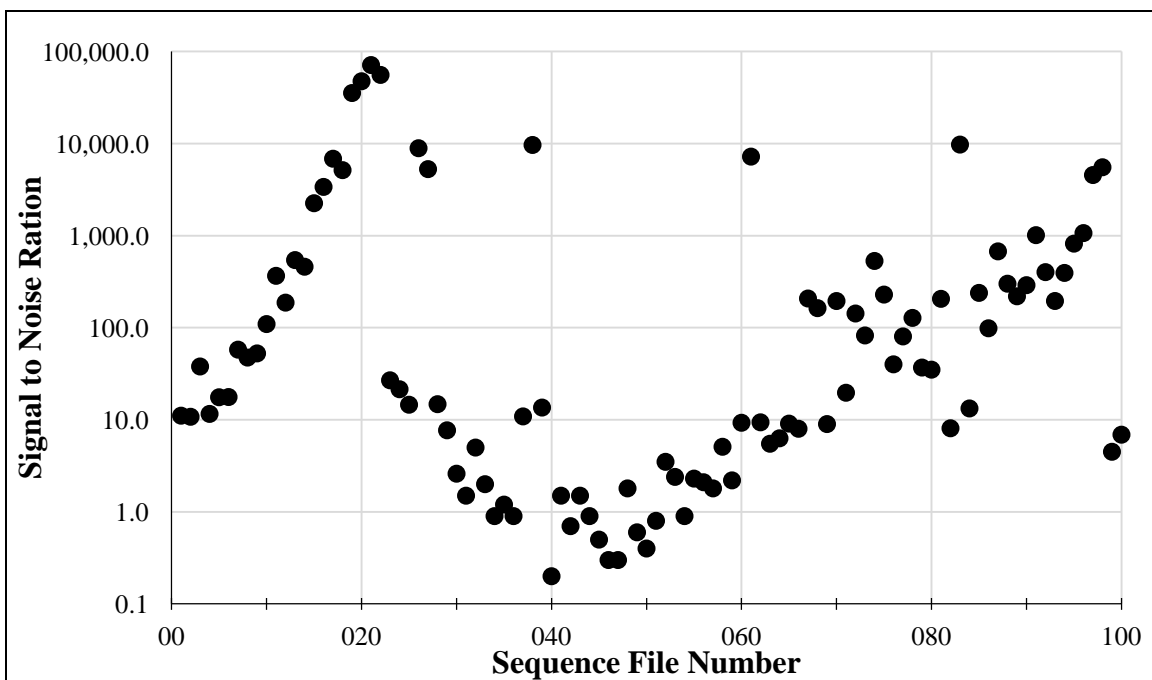


Figure 60: Batch Test 2 – Signal to Noise Ratio

## Appendix F: Batch Test 3 Results

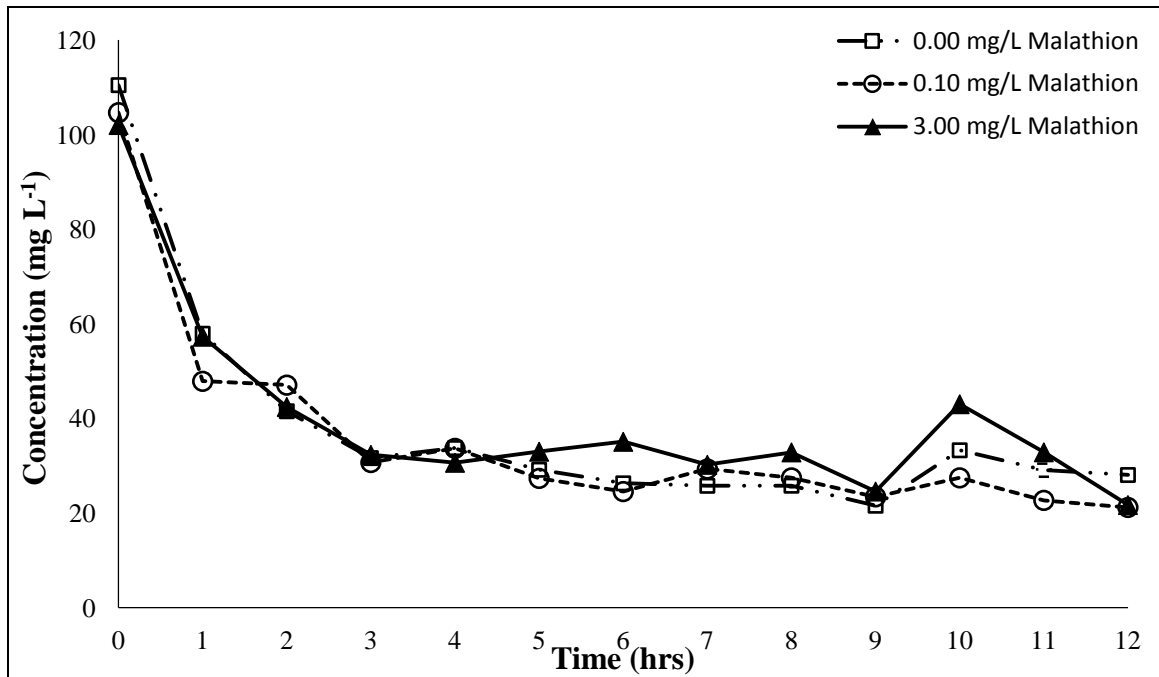


Figure 61: Batch Test 3 – COD Removal

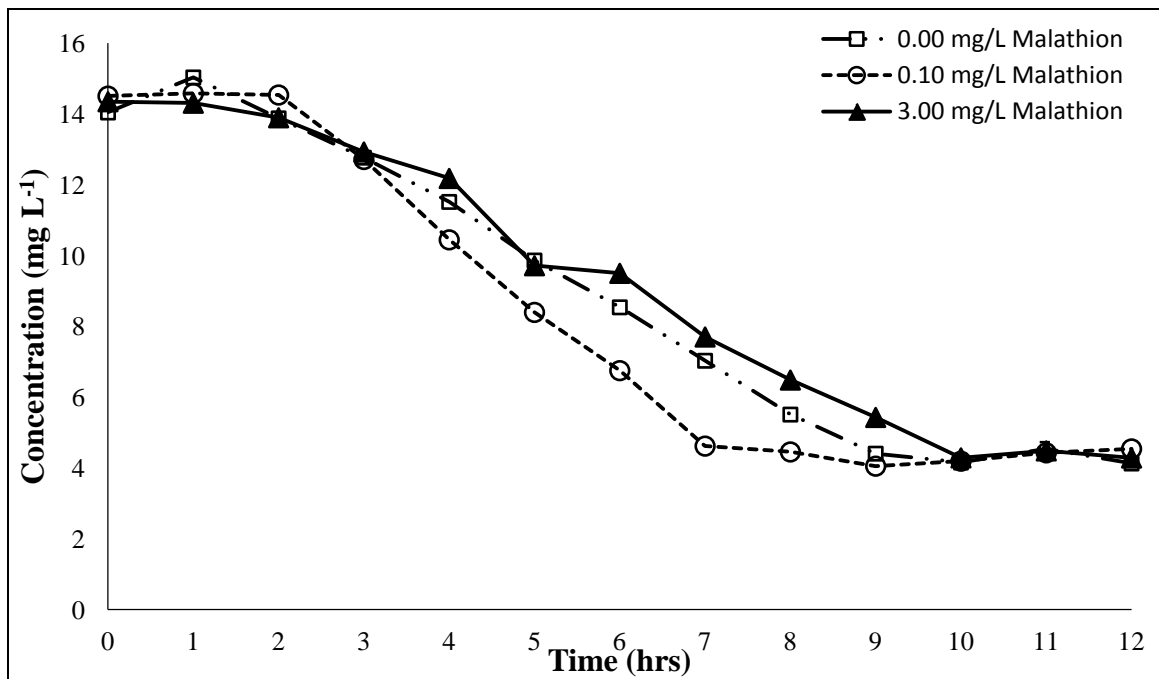


Figure 62: Batch Test 3 – NH<sub>3</sub> Removal

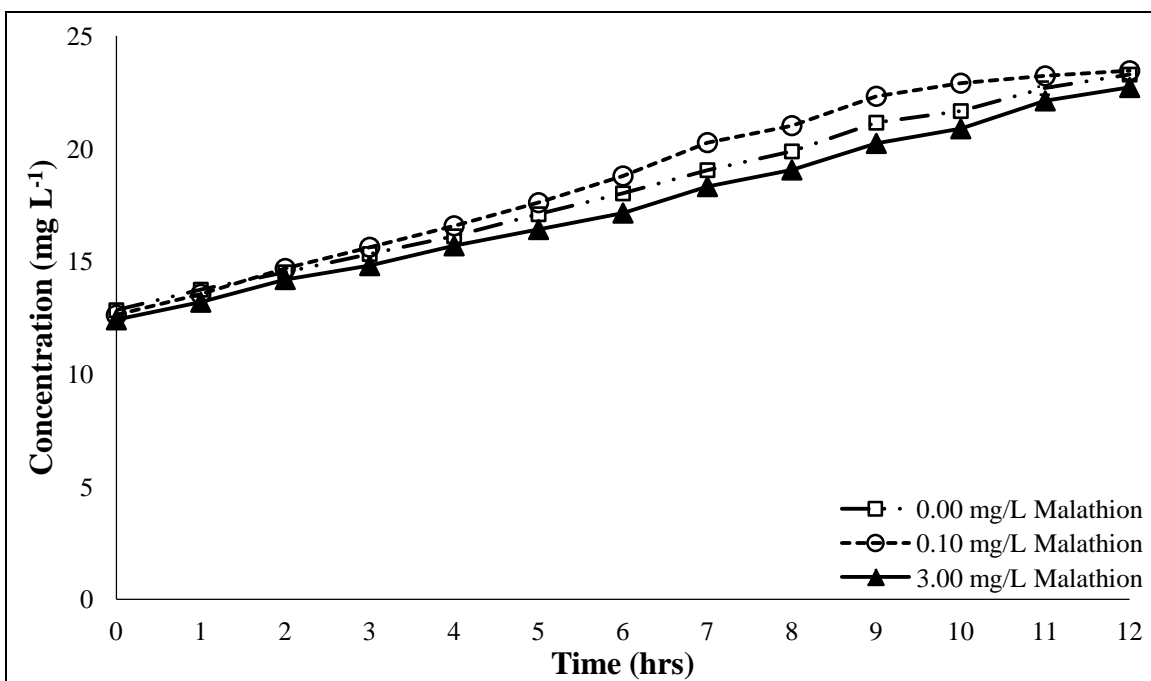


Figure 63: Batch Test 3 –  $\text{NO}_3$  Production

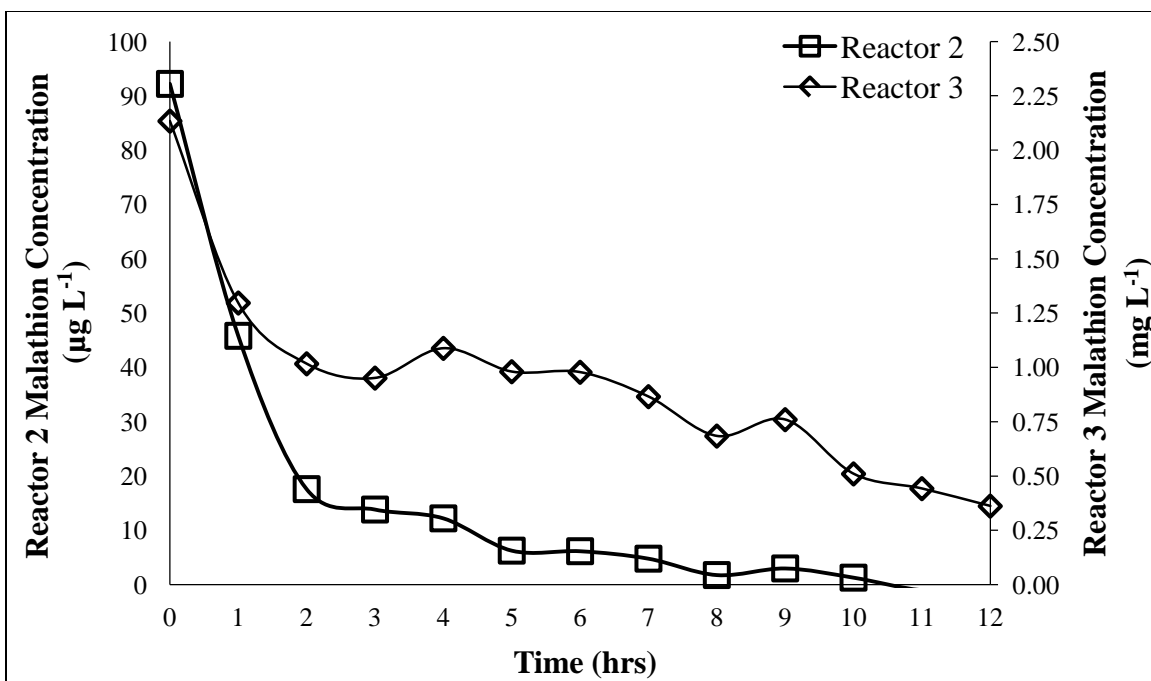


Figure 64: Batch Test 3 – Malathion Degradation



Table 15: Batch Test 3 – GC/MS Sequence Data File Reference																																			
File #				Concentration				File #				Reactor				Hour				Sample															
1				MeOH Blank				50				2				7				2															
2				MeOH Blank				51				2				7				2															
3				0.001 mg L <sup>-1</sup>				52				2				6				1															
4				0.001 mg L <sup>-1</sup>				53				2				6				2															
5				0.005 mg L <sup>-1</sup>				54				2				5				1															
6				0.005 mg L <sup>-1</sup>				55				2				5				2															
7				0.010 mg L <sup>-1</sup>				56				2				4				1															
8				0.010 mg L <sup>-1</sup>				57				2				4				2															
9				0.050 mg L <sup>-1</sup>				58				2				3				1															
10				0.050 mg L <sup>-1</sup>				59				2				3				2															
11				0.100 mg L <sup>-1</sup>				60				MeOH Blank				1.0 mg L <sup>-1</sup> Check Sample				100				MeOH Blank											
12				0.100 mg L <sup>-1</sup>				61																											
13				0.500 mg L <sup>-1</sup>				62				MeOH Blank				2				2				1											
14				0.500 mg L <sup>-1</sup>				63																											
15				1.000 mg L <sup>-1</sup>				64				2				2				2															
16				1.000 mg L <sup>-1</sup>				65				2				1				1															
17				5.000 mg L <sup>-1</sup>				66				2				1				2															
18				5.000 mg L <sup>-1</sup>				67				2				0				1															
19				10.00 mg L <sup>-1</sup>				68				2				0				2															
20				10.00 mg L <sup>-1</sup>				69				MeOH Blank				3				12				1											
21				MeOH Blank				70																											
22				H <sub>2</sub> O Blank				71				3				12				2															
23				MeOH Blank				72				3				11				1															
24				1.000 mg L <sup>-1</sup>				73				3				11				2															
25				1.000 mg L <sup>-1</sup>				74				3				10				1															
26				MeOH Blank				75				3				10				2															
27				MeOH Blank				76				3				9				1															
28				MeOH Blank				77				3				9				2															
				Reactor				Hour				Sample				78				3				8				1							
29				1				12				1				79				3				8				2							
30				1				12				2				80				3				7				1							
31				1				8				1				81				3				7				2							
32				1				8				2				82				MeOH Blank				1.0 mg L <sup>-1</sup> Check Sample				101				1.0 mg L <sup>-1</sup> Check Sample			
33				1				4				1				83																			
34				1				4				2				84				MeOH Blank				3				6				1			
35				1				0				1				85																			
36				1				0				2				86				3				6				2							
37				MeOH Blank				1.0 mg L <sup>-1</sup> Check Sample				MeOH Blank				87				3				5				1							
38																																88			
39				MeOH Blank				89				3				4				1															
40				2				12				1				90				3				4				2							
41				2				12				2				91				3				3				1							
41				2				11				1				92				3				3				2							
43				2				11				2				93				3				2				1							
44				2				10				1				94				3				2				2							
45				2				10				2				95				3				1				1							
46				2				9				1				96				3				1				2							
47				2				9				2				97				3				0				1							
48				2				8				1				98				3				0				2							
49				2				8				2				99				MeOH Blank				102				H <sub>2</sub> O Blank							

\* 2 Step dilution 100 mg L<sup>-1</sup> to 10 mg L<sup>-1</sup>, 10 mg L<sup>-1</sup> to 1 mg L<sup>-1</sup>

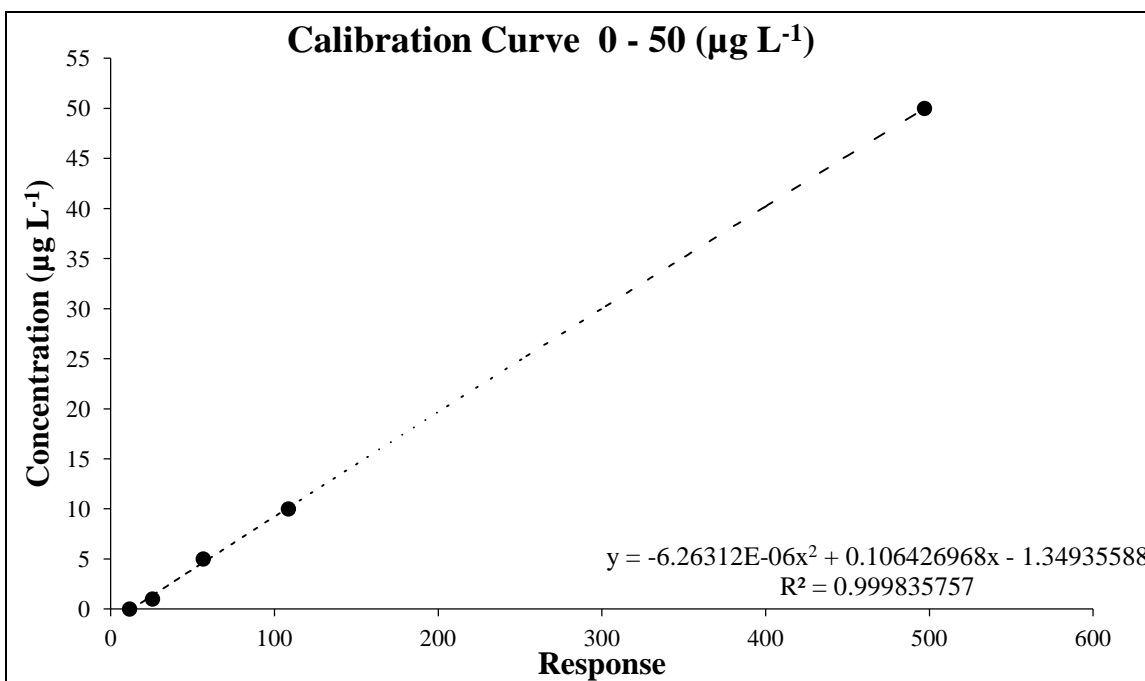


Figure 65: Batch Test 3 – Low Range Calibration Curve

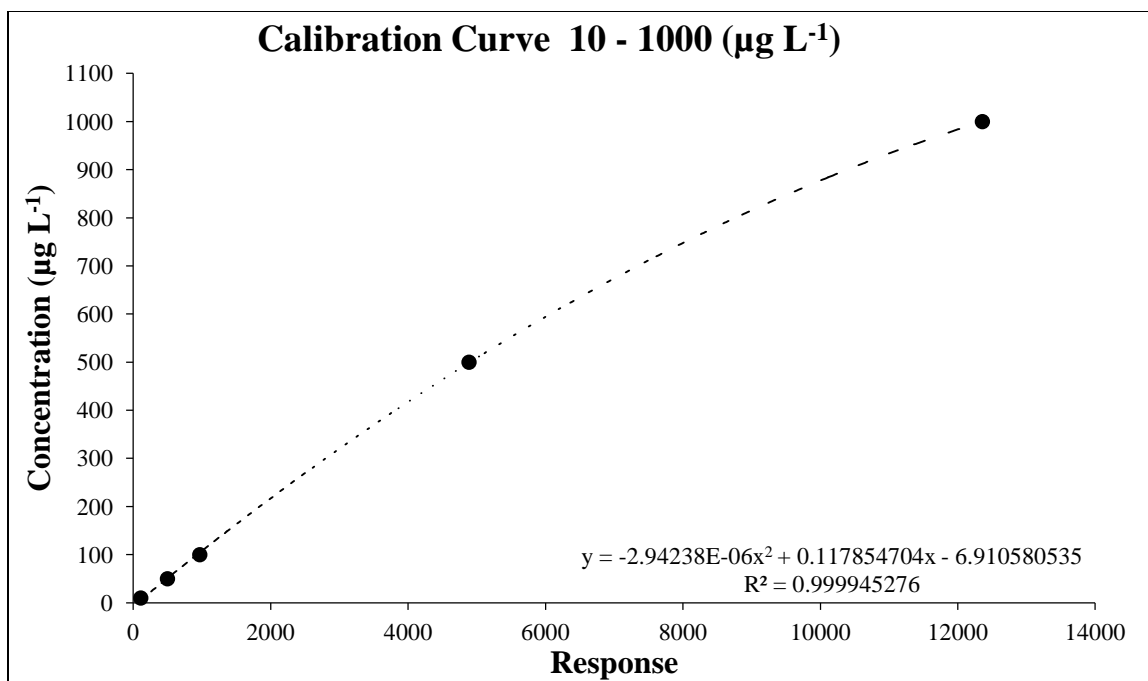
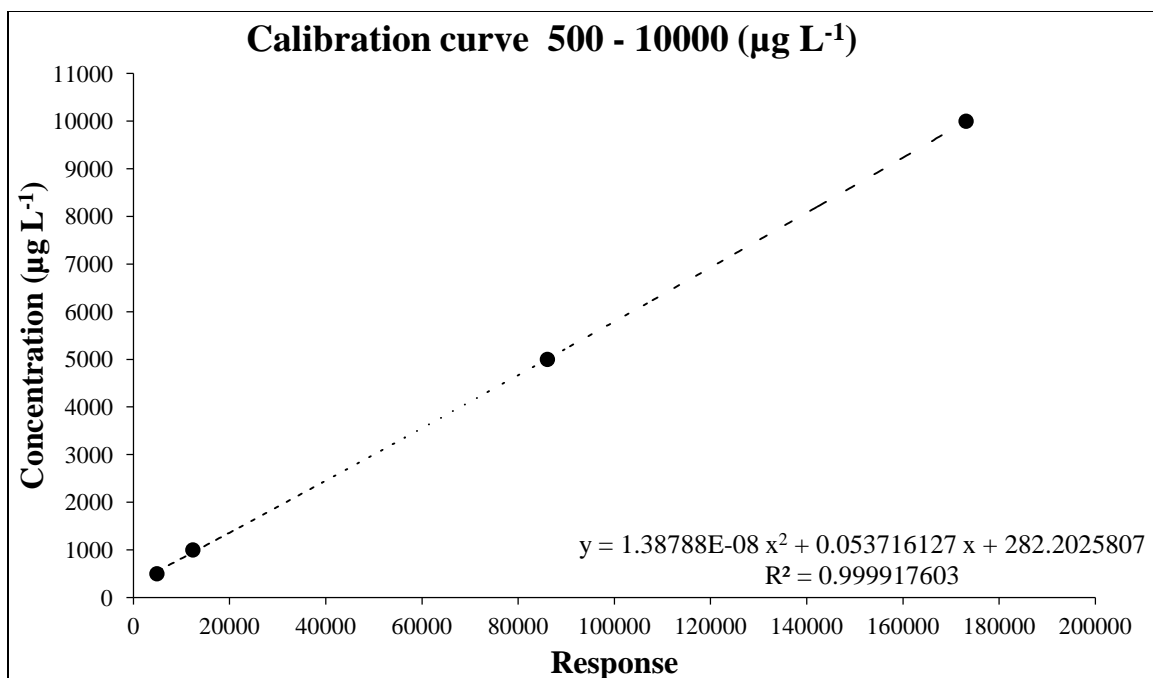


Figure 66: Batch Test 3 – Mid Range Calibration Curve



**Figure 67: Batch Test 3 – High Range Calibration Curve**

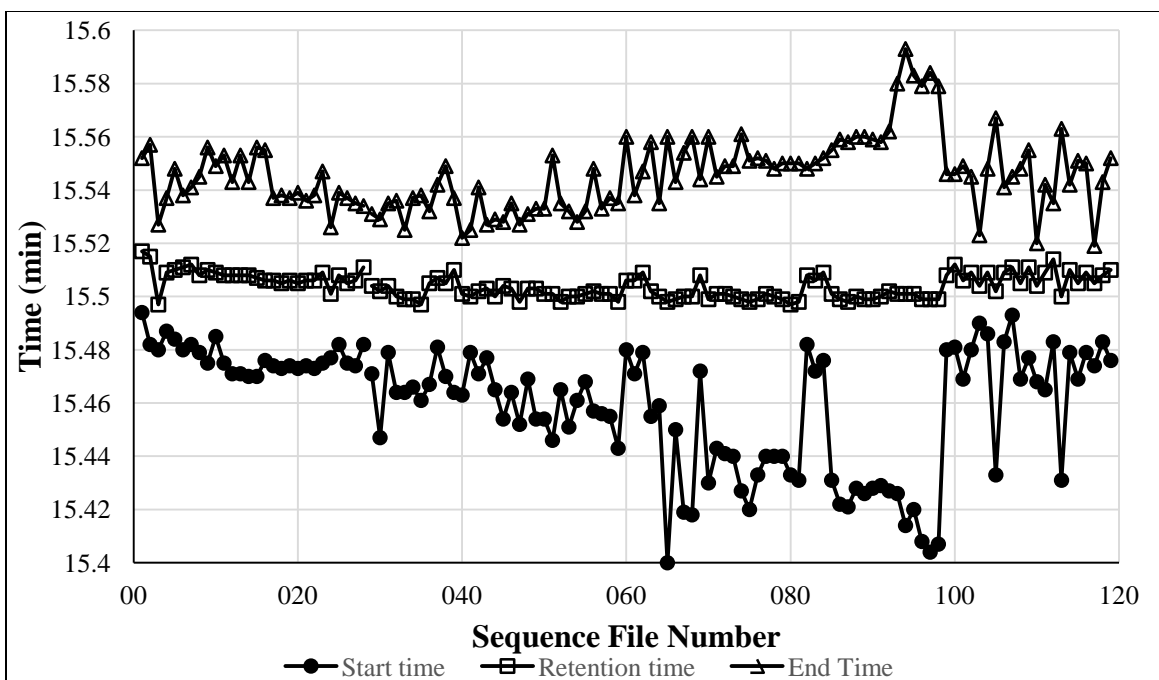


Figure 68: Batch Test 3 – GC/MS Retention Time Information

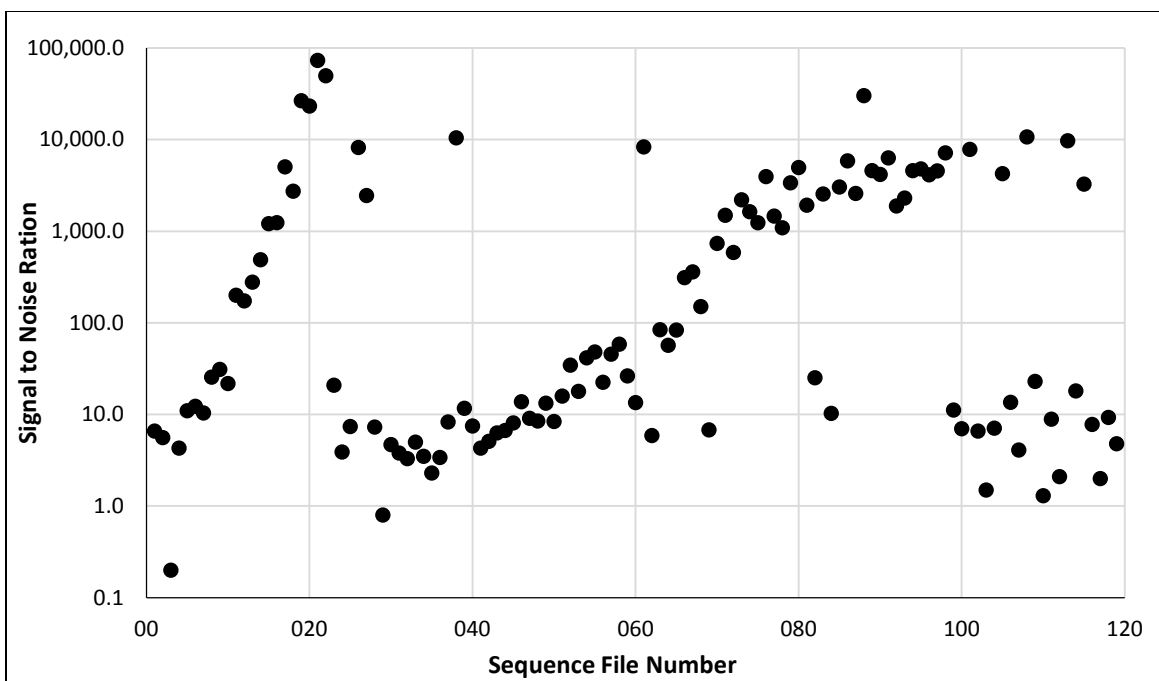


Figure 69: Batch Test 3 – Signal to Noise Ratio

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14. ABSTRACT: Decontamination activities following a chemical weapons incident may generate significant quantities of contaminated wash water that may enter the wastewater collection system either intentionally or accidentally. This raises concerns about the effect of chemical warfare agents (CWAs) on the operation of a biological wastewater treatment plant. The goal of the study was to evaluate the effect of malathion on the activity and performance of activated sludge. Malathion is an organophosphate (OP) and is considered an environmental surrogate for VX. This study employed respirometry, short term batch tests, and long term exposure experiments to investigate the effects of different concentrations of malathion on bioreactor performance. Short-term exposure to malathion may depress microbial respiration, however, short-term batch tests showed that chemical oxygen demand (COD) removal was not negatively impacted by the presence of malathion concentrations of 0.1 or 3 mg/L. Unlike COD removal, ammonia-N removal was slowed by the presence of malathion at both 0.1 and 3 mg/L with a positive correlation of the removal rate to the quantity of total suspended solids. Long-term exposure experiments demonstrated that both COD removal and Nitrate production were negatively affected at concentrations of 3.0 mg/L and unaffected at concentrations of 0.1 mg/L.					
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